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SOUTH PLATTE RIVER BASIN IRRIGATION OF VEGETABLES WITH SEWAGE-POLLUTED WATER

Federal Water Pollution Control Administration
Denver, Colorado

November 1967

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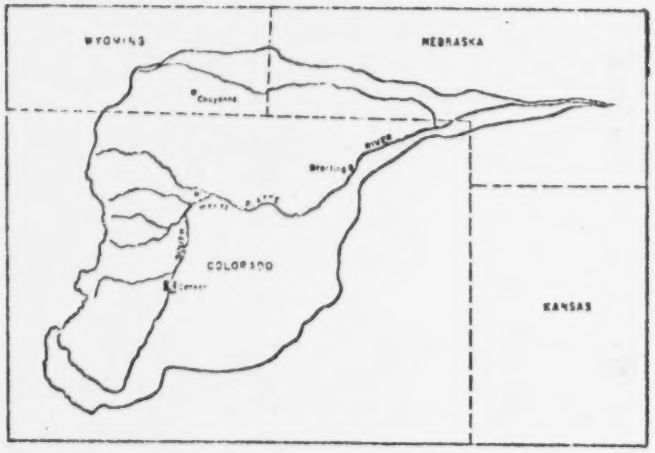
SOUTH PLATTE RIVER BASIN
IRRIGATION OF VEGETABLES WITH SEWAGE-POLLUTED WATER



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Denver, Colorado

November 1967

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SOUTH PLATTE RIVER BASIN

IRRIGATION OF VEGETABLES WITH SEWAGE-POLLUTED WATER

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U. S. DEPARTMENT OF THE INTERIOR
Federal Water Pollution Control Administration
South Platte River Basin Project
Denver, Colorado

November 1967

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SOUTH PLATTE RIVER BASIN PROJECT
Federal Water Pollution Control Administration

Background and Objectives

On July 18, 1963, the Governor of Colorado requested the Secretary of Health, Education, and Welfare to assist the State in determining sources of pollution and quality of waters of the South Platte River Basin within the State of Colorado.

The First Session of the Conference in the matter of Pollution of the South Platte River Basin was held in Denver, Colorado, on October 29, 1963, under the provisions of the Federal Water Pollution Control Act (33 U.S.C. 466 et seq.). It was recommended at this Conference that a study would be undertaken by facilities of the U. S. Public Health Service, now the Federal Water Pollution Control Administration, U. S. Department of the Interior. Findings and recommendations from this study were to lead to a program of pollution abatement to be developed jointly by the South Platte River Basin Project and the Colorado State Department of Public Health.

The long-range goals and objectives of the Project are as follows:

1. Determine the legitimate water uses and locate the sources of pollution having an adverse effect on those uses.

2. Through field investigations determine the physical, chemical, and biological responses of the River to pollution and evaluate the previously located sources of pollution with respect to the conditions in the River.

3. Compute the waste load reductions necessary to obtain desired water quality and recommend water quality control measures needed to effect the desired waste load reductions.

4. Recommend remedial time schedules for pollution abatement action.

The Second Session of the Conference was held in Denver, Colorado, on April 27-28, 1966, under the provisions of the Federal Water Pollution Control Act, as amended. At this Conference a report was made by the Project which included specific recommendations and time schedules for pollution abatement in the Denver Metropolitan area and for the sugar beet and cattle feedlot industries throughout the Basin. Specific water quality objectives were recommended for the South Platte River and its major tributaries.

The Second Session of the Conference was reconvened in Denver, Colorado, on November 10, 1966. At this Conference a pollution abatement schedule was adopted by the Colorado Water Pollution Control Commission and the Federal Water Pollution Control Administration.

Organization

The South Platte River Basin Project consists of engineering and associated professional and clerical personnel. The Project Headquarters offices are located in Denver, Colorado. The Project is administratively attached to the Missouri Basin Region, Federal Water Pollution Control Administration, U. S. Department of the Interior, Kansas City, Missouri.

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7. Boulder County Agricultural Extension Service, Longmont, Colorado

8. Denver County Agricultural Extension Service, Denver, Colorado

9. Jefferson County Agricultural Extension Service, Golden, Colorado

10. Weld County Agricultural Extension Service, Greeley, Colorado

11. Dr. Stuart G. Dunlop, Associate Professor of Microbiology, University of Colorado Medical Center, Denver, Colorado

SUMMARY AND CONCLUSIONS

The possible health hazard resulting from irrigation with sewage-polluted water of vegetables normally consumed raw has long concerned public health officials. Conflicting opinions have evolved on the degree of danger caused by this practice. Commonly accepted objectives for sewage irrigation practices have not been developed, nor have there been established generally approved standards for the quality of irrigation water.

In the South Platte River Basin downstream from and north of Denver to Brighton, Colorado, the largest use of water is for agriculture. In the area immediately north of Denver many varieties of "salad vegetables" were raised. Salad vegetables are those which are normally not peeled, pared, or cooked prior to eating. At the time of this study these vegetables were irrigated with polluted water diverted via four main supply ditches from the South Platte River just below entry of the Denver Northside sewage treatment plant effluent. Other inadequately treated municipal and industrial effluents contributed to the overall pollution in this vicinity.

Field observations of this portion of the South Platte River Basin conducted during this investigation revealed an unsightly appearance along the stream beds, unmistakably putrid odors, and obvious visual evidence of polluted water.

During 1963-64 a field sampling and laboratory analysis program was conducted on vegetables, the soils in which they were grown, and the waters with which they were irrigated. Farms using polluted water and another group irrigating with relatively clean water were sampled. Data were gathered on the physical and chemical characteristics of the irrigation water. Samples of vegetables, soils, and waters were analyzed for three bacterial indices of pollution -- confirmed, completed, and fecal coliforms. A total of 170 irrigation water, 143 soil, 75 root vegetable, and 115 leaf vegetable samples were tested.

The major emphasis in this study was placed on the fecal coliform group of bacteria because recently improved and standardized detection methods for fecal coliforms were available and this group is of primary sanitary significance. Since these organisms are characteristic of the intestinal tract and fecal excreta from humans and domestic animals, they are most likely to be associated with the various diseases transmitted in the fecal excreta of warm-blooded animals. Therefore, fecal coliform determinations yield valuable information on the possible sources, degree, and recency of pollution. The resulting data are most useful in the determination of the possibility of hazard from enteric and waterborne diseases.

Sewage irrigation practices in the Western United States and European countries have been summarized where they may be of value in providing guidelines for other irrigation areas. Examples of specific standards for irrigation water quality and objectives for sewage irrigation practices currently subscribed to by California, Missouri, Nebraska, and Utah have been cited.

The history of investigations concerning vegetable irrigation with polluted water in Colorado from 1933 to the present has been traced.

Completed as well as confirmed coliform values were used throughout to report the total coliform group in order to eliminate false positive results which could be expected to originate in soil and on vegetation.

The irrigation water diverted from the South Platte River immediately downstream from Denver in the Burlington-O'Brian, Gardeners, Fulton, and Brantner ditches was found to be grossly polluted. The geometric mean values for the confirmed, completed, and fecal coliform densities in the irrigation waters were: 420,000, 290,000, and 58,000 MPN/100 ml respectively. The bacterial results demonstrated that as the quality of the irrigation water deteriorated, the coliform group densities increased.

There was ample proof that fecal microorganisms in various concentrations found their way from the irrigation water to root crops and leaf vegetables. Evidence verified that the fecal coliform bacteria were present in greater numbers in soils and on vegetables irrigated with polluted water. The total (completed) and fecal coliform geometric mean densities were significantly higher for the farms irrigating with polluted water than for those using comparatively pure water with the exception of leaf vegetables. The difference between fecal coliform levels for leaf vegetables irrigated with polluted and unpolluted water was negligible.

The vegetable sample results were divided into two categories, soil contact (root) crops with edible portions grown below the soil surface, and leaf crops with the edible parts grown above ground. The root crops demonstrated higher coliform densities than the leaf vegetables. Furthermore, market root vegetables exhibited higher coliform values than field root crops. This observation reflected additional contamination of market root vegetables due to washing with polluted water, bunching, handling, and other market procedures. Leaf vegetables usually trimmed but not washed or bunched did not show this difference in market versus field crops.

The presence of fecal coliform bacteria on vegetables usually eaten raw indicates a potential public health hazard. Proof of coliform bacteria of fecal origin implies association with disease-causing organisms. As the numbers of bacteria typical of fecal pollution increase, the relative probability of hazard from enteric diseases increases.

A brief survey was conducted during part of the growing season for the presence of enteric pathogens on the vegetables, in the soils, and in the irrigation waters. Salmonella were recovered from eleven water samples, one recently irrigated soil sample, and no vegetable samples.

Other investigations into the survival of enteric organisms in soil and water and on vegetables have been reviewed as a part of this study.

RECOMMENDATIONS

Water of highest attainable quality should be used for irrigation of vegetables consumed raw. Where polluted water is used in the absence of standards or regulations and clean water cannot be supplied, interim objectives should be adopted. In view of the results of this study and the observations of other investigations, the following recommendations for sewage irrigation of vegetables are made in the interest of sound public health practices:

1. Sewage effluents above the points of diversion for the irrigation area should receive adequate secondary treatment and disinfection.

2. Irrigation with polluted water should cease at some reasonable period of time before harvest of vegetables and unpolluted water should be used thereafter. Additional studies should be undertaken to determine the logical cessation time in this area.

3. The farm operators should wash vegetables with constantly flowing water conforming to USPHS Drinking Water Standards.

4. Farmers, field laborers, and produce handlers working with or near sewage pollution should be instructed in sanitary hygiene for their own protection, and the improvement of the sanitary quality of their produce.

5. The responsible public health agencies must develop and enforce water quality criteria for irrigation of vegetables normally consumed raw and definite objectives for sanitary irrigation practices.

6. Regular unannounced field surveys and adequate bacteriological control measures should be instituted by the regulatory agencies to monitor farming and marketing procedures.

7. An epidemiological study of the area would be advisable to determine the actual hazard of transmitting disease-causing enteric organisms by the present sewage irrigation practices.

INTRODUCTION

The rapidly growing population and industrial expansion in the Denver area have resulted in an accompanying increase of pollution in the South Platte River. Large volumes of South Platte River water carrying considerable amounts of untreated or inadequately treated sewage were being used at the time of this study for irrigation of truck crops that are normally consumed raw. This possible health hazard has been a problem of concern to public health authorities for many years.

In the letter from Governor Love dated July 18, 1963, (1) to the Secretary of Health, Education, and Welfare, it was stated that "serious pollution problems exist in Adams County" and specifically mentioned that "information is necessary in planning for the water demands relating to . . . . . irrigation . . . . . and other uses, most of which require water of high quality." In the First Session of the Conference in the matter of Pollution of the South Platte River Basin (2) it was further stated that "this problem of bacteriological contamination is most critical in the farming area downstream and north of Denver. Further study is urgently needed in this regard." A general map of the South Platte River Basin is shown in Figure 1.

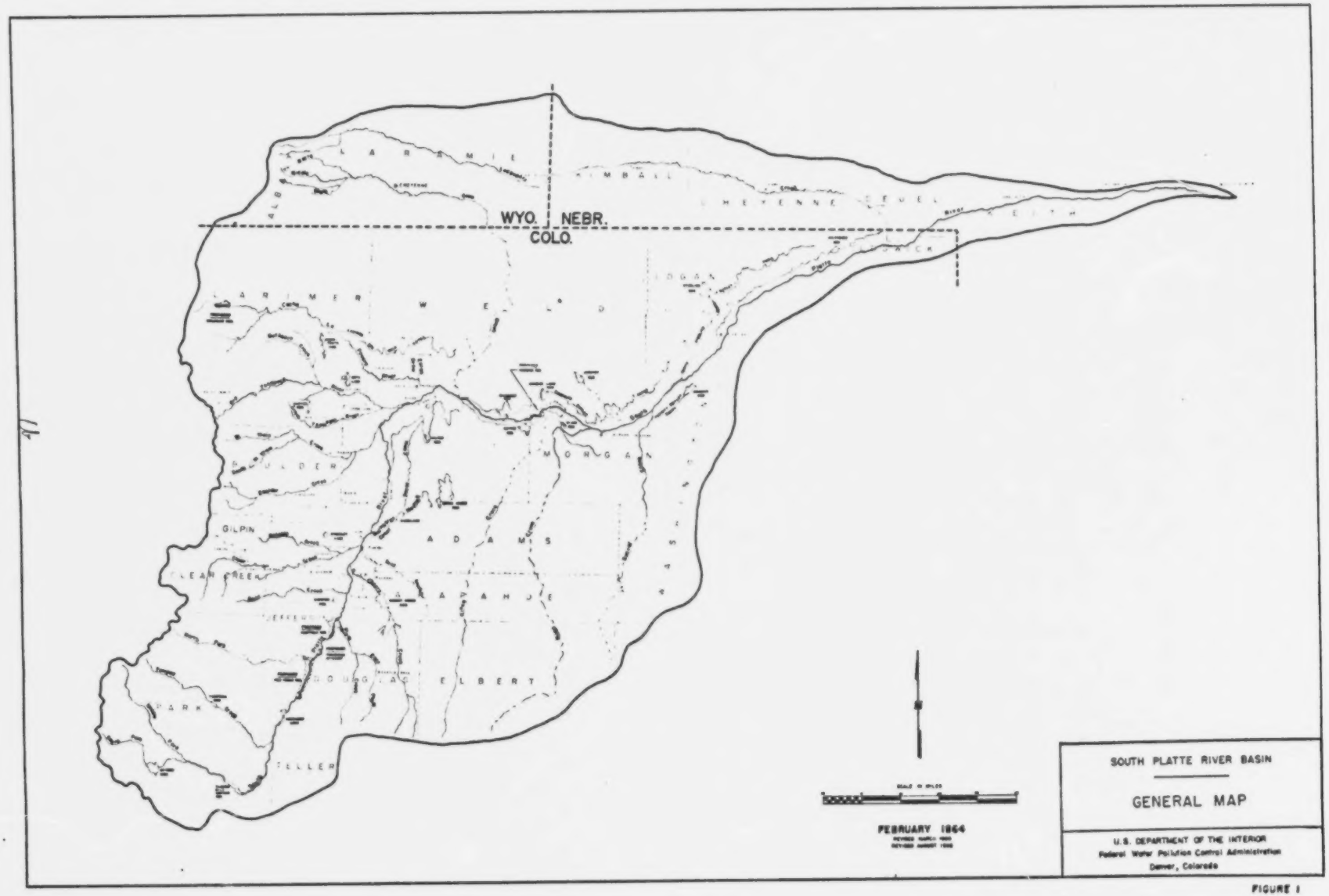
The study began with a review of the public health aspects of irrigation with sewage-contaminated water. A field reconnaissance of the vegetable farm region within the Denver Metropolitan area of the South Platte River Basin using polluted water followed. Other truck farms using relatively clean water were observed in the vicinity where all known environmental factors such as climatic conditions, irrigation practices, and types of crops raised were similar. Figure 2 depicts a field worker irrigating vegetables in the South Platte River Basin. A sampling program was planned for vegetables, the soils in which they were grown, and the waters with which they were irrigated.

The objectives of the study were:

(a) To further evaluate the hazard of consuming raw vegetables irrigated with sewage-polluted water.

(b) To compare bacterial levels on vegetables irrigated with polluted irrigation water to those grown with relatively unpolluted water.

(c) To ascertain the types of vegetables most susceptible to contamination.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YUMA
WASHINGTON
FEBRUARY 1964
SOUTH PLATTE RIVER BASIN
GENERAL MAP
U.S. DEPARTMENT OF THE INTERIOR
Federal Water Pollution Control Administration
Denver, Colorado

FIGURE 1

(d) To determine the effect of the marketing procedures upon the bacterial quality of the truck crops, a

(e) To find the current levels of pollution on the vegetables, in the soils, and in the irrigation waters.

Preliminary bacteriological investigations were performed by the Public Health Service during the latter part of the growing season in September 1963. After analysis of the 1963 data, the indications were that a potentially serious problem existed. The South Platte River Basin Project, Tri-County Health Department and the farmers concerned met on several occasions in the Spring of 1964. Full-scale field investigations were conducted during the growing season from April 25 to September 28, 1964.

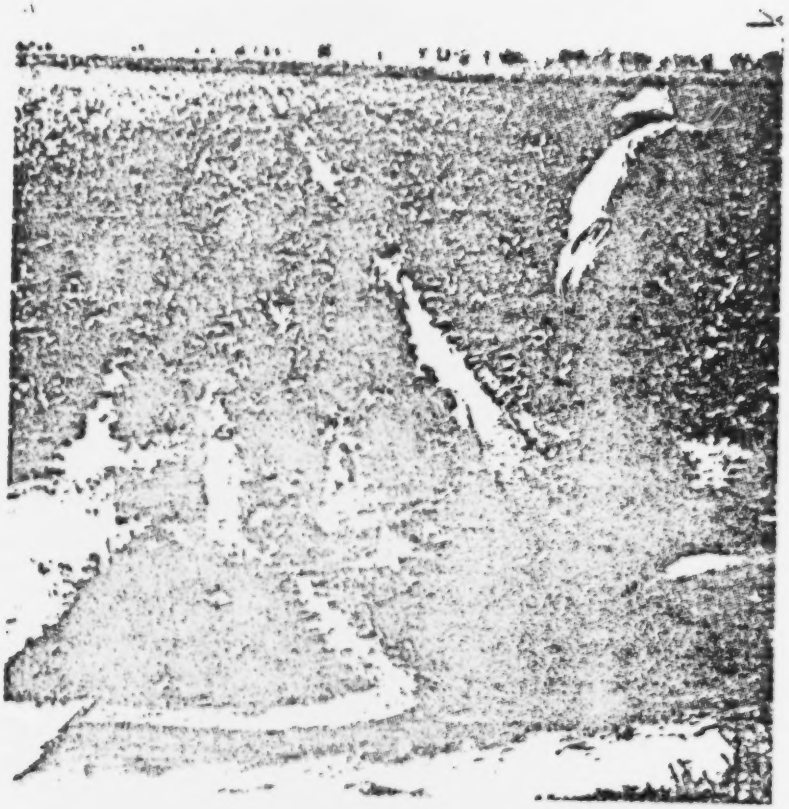

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Figure 2
Vegetable Irrigation in the
South Platte River Basin

DESCRIPTION OF THE AREA

The portion of the South Platte River Basin included in this investigation is shown in Figure 3. The area in which truck crops were irrigated with polluted water is located in the bottomlands of the Basin, primarily between North Denver and Brighton, Colorado, in the western part of Adams County and the southwestern portion of Weld County. Lesser acreages of vegetables irrigated with polluted water remained in Denver County along the River and in Jefferson County along Clear Creek. Figure 4 typifies an irrigation ditch in the South Platte River Basin carrying sewage-laden water. The foam below the small dam and drop structure is due to detergents of sewage origin.

Large quantities of water were used on the farms in this region during the growing season. The chief source of polluted irrigation water was the South Platte River which flows to the north and northeast after passing through the City and County of Denver, which in July 1964 had a population of 525,000 according to the State Commerce and Development Division, with the Greater Metropolitan area reaching a population of over 1,000,000. The water was diverted from the South Platte River via four major irrigation canals; the Burlington-O'Brian; Gardeners; Fulton; and Brantner Ditches, which take off approximately 0.2, 0.9, 8.0, and 10.2 miles respectively below the waste discharge outlet of the Denver Northside Sewage Treatment Plant. The Northside Plant is adjacent to the Denver-Adams County Line.

At the time of the study, heavy pollution was contributed to the South Platte River by the inadequately-treated effluent of the Denver-Northside Plant and municipal-industrial wastes upstream of the study area. Further pollution was added by industrial outfalls and partially-treated municipal sewage from East Denver and Aurora carried by Sand Creek and large amounts of inadequately-treated sewage in Clear Creek. Packing plant effluents in the area added their wastes to the irrigation water. This situation was aggravated by low flow conditions throughout.

There were approximately 5700 acres and 310 farms devoted to vegetable growing under irrigation and utilizing polluted water in the study area (Adams,Denver, Jefferson, and Weld Counties), according to estimates by the Agricultural Extension agents of these counties. The farms varied in size from 2 to 3 acres to larger ones up to 100 acres. The Vegetable Producers Cooperative Association of Colorado, to which about 95 percent of the vegetable farmers in the Denver-Brighton area belonged, had 300 members during the 1963-64 seasons and handled truck crop vegetables reaching a wholesale value of $768,000 during the 1963 and $837,000 during the 1964 seasons. It was estimated (3) that at least 75 percent of these were shipped out of the State, subjecting them to Interstate Commerce regulations.

The agricultural areas utilizing comparatively clean water were in the same geographical region. The control farm sampled in 1963 was situated adjacent to Bear Creek in Jefferson County immediately southwest of the City and County of Denver. Its source of irrigation water was seepage from Hodgson and Warrior Ditches. The area irrigating with relatively pure water included in the 1964 study lies west of Longmont, Colorado, in the southeastern section of Boulder County. The irrigation water for the farms sampled in this area was taken from the Lefthand, Niwot, Rough and Ready, and Swede Ditches. These ditches were in turn diverted from Lefthand and South St. Vrain Creeks which had little contact with populated areas.

The climate of this region is best described as sunny, mild, and semi-arid with 14.8 inches of mean annual precipitation according to the Weather Bureau, U. S. Department of Commerce. On the average there are 115 clear days and 135 partly cloudy days during the year. Seventy percent of the possible sunlight hours are sunlit, providing an abundance of ultra violet light. The elevation is 5,000-5,200 feet above sea level. The mean average monthly temperature from May through September, the normal growing season, ranges from 56.7 to 72.6 degrees.

This dry, sunny climate provides good growing and harvesting weather but also causes vegetable growers to rely almost entirely upon heavy irrigation throughout the growing season. The ratio of ground water to surface water used ranges from 30 to 50 percent. Most vegetable growers supplement the surface supply with well water. However, many of the smaller farmers rely almost entirely upon the surface water supply. The large majority of farm operators use the furrow method of irrigation, allowing the water to flow through the furrows between planted rows. Figure 5 portrays furrow irrigation in a Colorado field.

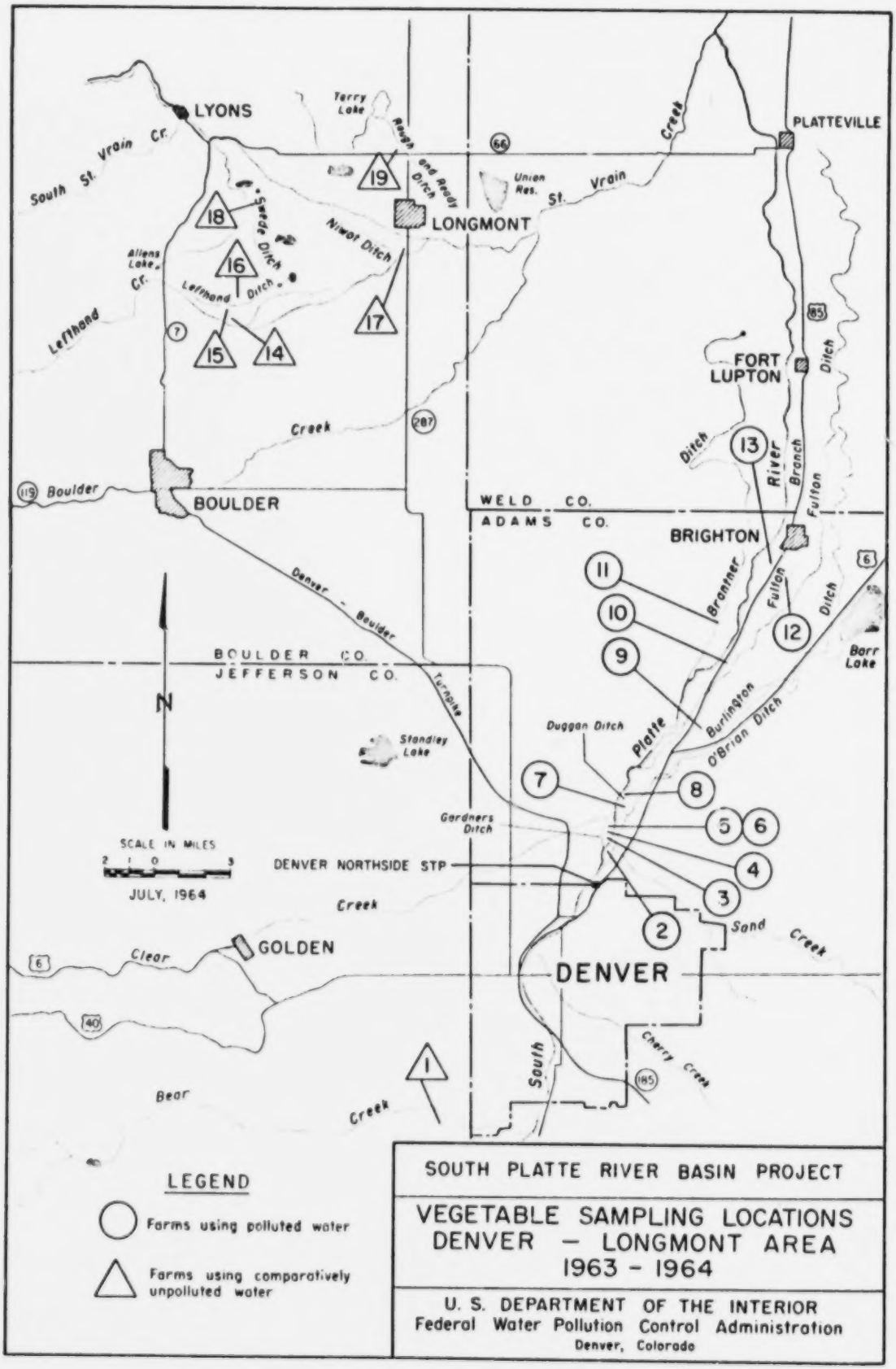

FIGURE 3

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Figure 4
Irrigation Ditch in the South Platte River Basin carrying sewage-polluted water. Note the detergent-caused foam.

Figure 5
Furrow Irrigation in Colorado

BACKGROUND

Sewage Organisms

Polluted irrigation water may serve to transmit a wide range of organisms onto land or crops. Some of these organisms are capable of causing disease and others serve as indicators of sewage contamination such as coliform bacteria.

Since the edible portions of fresh vegetables are grown in or near the ground, they have an excellent opportunity to become contaminated by soil or water-carried organisms or by insanitary handling and market preparation, including the use of polluted water to rinse or "freshen" the product.

The pathogenic organisms which may be borne by sewage or contaminated water include enteric bacteria producing gastroenteritis, bacillary dysentery, diarrhea, and salmonellosis; protozoa; possibly viruses, including infectious hepatitis; and other parasitic infestations. Leptospirosis or tubercule bacilli are other pathogenic entities which may be carried by water and sewage. Human infection by these pathogens may occur either by consumption of contaminated food or water or through swimming, bathing, or direct contact with polluted water.

Typhoid fever caused by Salmonella typhi, once a commonly feared enteric disease, is now relatively rare in this country. However, other pathogenic members of the Salmonella group cause similar enteric disorders and there are indications that non-typhoid salmonellosis is increasing in the United States. These diseases are more insidious because many are not easily recognized and frequently go unreported when they are evidenced only as mild intestinal upsets or indigestion. Infants and senile persons are most susceptible and fatalities sometimes result in these groups. The causative organisms are carried and excreted by animals as well as humans. Salmonellosis, therefore, can create a hazard in sewage and sewage-polluted water, and on vegetables and other foods in contact with these contaminated materials. The hazard increases in proportion to increasing human and animal populations and their case and carrier rates.

Pollution Indicators

Indicator organisms of pollution are used because practical routine quantitative or semi-quantitative procedures for enteric pathogenic bacteria are not available. Techniques for the isolation and enumeration of pathogens are cumbersome, time-consuming, and lack quantitative significance. When evidences of indicator organisms are found, they indicate sewage pollution and imply association with pathogens. The larger the number of pollution indicators found, the greater the possibility of hazard to public health.

Coliform bacteria have long been accepted as evidence of sewage pollution in water supplies and form the present basis for bacteriological water quality standards. They are always present in the intestinal tract of man and other warm-blooded animals and are excreted in large numbers in fecal wastes. Their density in water is roughly proportional to the degree of pollution and many times greater in sewage and water than the enteric pathogens. Members of the coliform group are widely distributed in nature as well as in the intestinal tract and under some circumstances coliform bacteria may be present which are derived from sources other than fecal excreta. Some microorganisms in the soil and on vegetables may yield false positive data by the "Confirmed Test" method. The "Completed Coliform Test" method is used to verify confirmed test results, thus eliminating false positives due to non-coliform microorganisms (4) and allowing greater accuracy in the enumeration of the total coliform density.

The fecal coliform test based upon growth and gas production by the fermentation of lactose at an elevated temperature eliminates coliforms derived from sources other than the gut of warm-blooded animals. It was shown (5) to have a 95 percent correlation with coliforms from fecal sources. This technique yields an index for that portion of the coliform group which is of fecal origin, and is therefore a very useful measuring device for estimation of fecal pollution in water and particularly on plants and in soil where large numbers of non-fecal coliforms may occur. Fecal coliform levels may divulge valuable information on the possible sources of pollution and the remoteness of the pollution time. There are no means available for the direct differentiation of coliforms of animal or human origin.

METHODOLOGY

Farm Sampling

The locations of the vegetable farms sampled are superimposed on the map of the study area in Figure 3 where they are shown in relation to their respective irrigation ditches, the South Platte River, and the Denver-Brighton-Longmont area. The twelve farms irrigating with polluted water are designated by sample station numbers 2 through 13 and encircled. The triangles numbered 1 and 14 through 19, represent the seven farms using comparatively clean water. Vegetables, soil, and the water with which they were irrigated were sampled at each farm.

Seven farms in the area north of Denver irrigated with polluted water were sampled late in the 1963 growing season. Farm No. 1 using relatively pure irrigation water, located immediately south of Bear Creek and southwest of Denver, was designated the control farm for the 1963 survey. In 1964 eight farms in the vicinity north and east of Denver using polluted irrigation water were selected. Three of the farms in this area sampled in 1963 were included in the 1964 group. Six control farms (Nos. 14 through 19) were chosen in 1964 and are located in the Longmont, Colorado area. These six control farms were served by four ditches whose waters originate in the mountains with little intervening pollution.

Detailed descriptions of each farm denoting the vegetables sampled, dates of sampling, source of irrigation water, and other pertinent information are listed in Appendix A.

Irrigation Water Samples

The irrigation waters at selected points on the ditches and at the headgate for each study farm were sampled weekly and analyzed for confirmed, completed, and fecal coliforms, biochemical oxygen demand (BOD), dissolved oxygen (DO), conductivity, and pH. Temperatures were recorded at the time of sampling. The bacteriological samples were taken in sterile 250 ml, glass-stoppered bottles, using a holder that was lowered below the water surface. All samples were immediately iced. The BOD samples were collected in one-liter plastic bottles and were then iced. The DO samples were secured with a "Sargent" sampler that housed a 300 ml, glass-stoppered bottle. The samples were fixed to the acid stage in the field and iced. The chemical samples were obtained in one-gallon plastic containers; these samples were not iced. Analyses were performed within six hours after the samples reached the Laboratory.

Irrigation waters examined for the presence of enteric pathogens were sampled by a gauze pad technique (6). A nine-inch wide bolt of gauze was folded into a fourteen-inch length of five thicknesses, then cut lengthwise into approximately 1-3/4 inch parallel strips to within

four inches of one end, resulting in five sets of 1-3/4 inch strips attached to the uncut end. The uncut end was then rounded and securely tied with cord or wire, forming a head with the gauze strips hanging free. The device was placed in the stream at the sample station just below the surface with the strips suspended. The head was tied to one or more stationary objects along the bank and anchored by a weight suspended from it in order to maintain a steadfast position. After five days this sampling device was retrieved, placed in a plastic bag, iced, and returned to the Laboratory.

Vegetable Wash Water

The water from a large wash tank typical of those used in the area for rinsing vegetables was sampled. The wash tank served a group of farmers growing crops in a cultivated area of several acres for rinsing large quantities of harvested root vegetables. The concrete tank was constructed to allow a continuous flow of water through its interior. Both the inflow and outlet drain waters were sampled.

Soil Samples

Soil samples were collected at selected time intervals from the plots where the vegetables were sampled. The soil was sampled one to two inches below the ground surface within a two-foot radius of the plant sampled, using a sterile spoon, and placed in a sterile plastic bottle.

Vegetable Samples

Sampling of vegetables was restricted to those which are likely to be eaten uncooked. They included beets, cabbage, carrots, cauliflower, celery, collard greens, endive, lettuce, green onions, mustard greens, parsley, peppers, radishes, turnips, and turnip greens.

Field vegetable samples were obtained by first dropping a sterile plastic bag over the plant and then pulling the entire plant from the ground (e.g. carrots, radishes, and green onions), or cutting off the stem with a sterile scalpel (cabbage or lettuce).

Preparations by the farmer for marketing of vegetables in this area included harvesting such crops as cabbage, cauliflower, celery, endive, and lettuce; trimming off the outer leaves; packing in crates; and spraying with water to prevent wilting. Carrots, green onions, radishes, and parsley were hauled from the fields, bunched, dipped in a tank of water to remove soil, and crated. Market vegetable samples were collected from these crates and placed in sterile plastic bags.

All vegetable samples were iced until they reached the Laboratory.

Laboratory Methods

Edible portions of the vegetables were removed aseptically and weighed. Non-edible portions, for example, the outer skin of green onions, blemished outer leaves of cabbage and lettuce, and the root structure of onions and radishes were not included. The loose soil clinging to root crops was scraped off aseptically before removing the outer layer of the vegetable itself. The remaining outer surfaces more likely to have been in direct contact with the handlers, marketing processors, or the immediate environment were tested. Twenty-gram samples of each vegetable and soil were blended in a Waring blender with 180 ml of buffered sterile water at high speed for 30 seconds. These blended samples and irrigation water samples, as received in the Laboratory, were promptly inoculated into the appropriate media and examined by the confirmed, completed, and fecal coliform multiple tube techniques. During the 1963 survey the completed coliform test was not performed on water samples. The most probable number (MPN) tests were based upon five replicate tubes at each of three significant dilutions. The confirmed and completed tests were as described in Standard Methods for the Examination of Water and Wastewater, 12th Edition (4) and the fecal coliform test was accomplished by confirming all positive presumptive tubes in EC broth incubated at $44.5 \pm 0.5^{\circ}C$ for 24 hours (5,7).

The tests for enteric pathogens were performed on portions of gauze strips which had been suspended in irrigation streams or ditches for five days, soil samples, and the outer edible surfaces of vegetable samples removed aseptically and cut with sterile scalpel or scissors into pieces small enough to be inserted into large-mouth flasks. The flasks contained enrichment media for enteric pathogens (Tetrathionate and SBG Sulfa Enrichment Broths) in 300 ml volumes. The inoculated enrichment media were incubated at $41.5^{\circ}C$ for 24 hours. Growth from these flasks was then streaked on each of four plating media selective for enteric pathogens--Brilliant Green, Bismuth Sulfite, "SS" (Salmonella-Shigella) and MacConkey agars--and incubated at $41.5^{\circ}C$ for 18 hours. Isolated colonies suspected of being enteric pathogens due to their characteristics and morphology on these selective media were picked and inoculated into a series of media for biochemical and serological reactions to verify that they were Salmonellae or Shigellae (8).

The possibility of inhibition of fecal coliforms by the blended vegetables was investigated. Blank paper discs were saturated with the blended material and placed along with selected antibiotic sensitivity control discs on pour plates heavily seeded with a typical fecal coliform culture. After 24 hours incubation these plates were read for zones of inhibition surrounding the various discs.

RESULTS

The coliform densities found for fifteen types of truck vegetables, the various soils in which they were grown and the water with which they were irrigated in the South Platte River Basin downriver from Denver have been summarized in Table I. The results from farms using polluted irrigation water have been listed separately to provide a comparison with those using relatively clean water. These data represent a total of 190 vegetable, 143 soil, and 170 water samples.

Although completed and fecal coliform data have been more closely associated with danger to public health, the "Confirmed Test" results have been included in the Tables for comparison purposes because many earlier investigations have not used the more accurate "Completed Test" procedure. The reduction of total coliforms from confirmed to completed results was from 1/5 to 1/200 for soil and vegetable samples due to the large numbers of false positives, whereas the decrease was only 2/3 to 1/2 for irrigation waters.

It is obvious from inspection of Table I that irrigation water derived from ditches which were diverted from the South Platte River were heavily polluted, yielding mean MPN values of 290,000 completed, and 58,000 fecal coliforms. By comparison, 2,100 and 630 were the respective mean MPN values for the area using clean irrigation water.

The coliform levels of the soils and vegetables reflected those of the waters with which they were irrigated. The mean completed coliform MPN was 55,000 and the fecal coliform MPN 3,200 for soils irrigated with polluted water, as contrasted to 530 and 32 for those irrigated with better quality water.

The coliform levels of the vegetables were lower than the soils in which they were grown. The data for vegetables reported in Table I have been divided into two groups: (a) root crops which include soil-contact vegetables that have their principal edible portion grown below ground, such as beets, carrots, radishes, turnips, and green onions; and (b) leaf vegetables consisting of those with their edible portion grown above ground--cabbage, lettuce, collard greens, turnip greens, mustard greens, cauliflower, celery, endive, peppers, and parsley. For root vegetables, the mean completed coliform MPN was 13,000 and the fecal coliform MPN 260, as opposed to 250 and 18 for those root crops irrigated with relatively clean water. Leaf vegetables gave values considerably lower in both categories.

These two vegetable groups (root and leaf) were further subdivided into field and market categories. Their coliform densities are shown in Table II. Field samples were taken directly from the vegetable plots irrigated with polluted water and the market samples were collected after

TABLE I

Coliform Densities of Vegetables, Soils, and Irrigation Water
Geometric Mean and 90th Percentile Distribution
of MPN Values per 100 ml or 100 Grams

Sample	No. of Samples	Confirmed Test		Completed Test		Fecal Test	
		Mean	90th%	Mean	90th%	Mean	90th%
Farms Using Polluted Irrigation Water							
Water	117	420,000	18,000,000	290,000*	12,000,000*	58,000	3,600,000
Soil	108	360,000	13,500,000	55,000	2,600,000	3,200	200,000
Root Vegetables	57	350,000	22,000,000	13,000	4,400,000	260	70,000
Leaf Vegetables	80	10,000	1,300,000	1,000	190,000	16	2,900
Farms Using Comparatively Unpolluted Irrigation Water							
Water	53	4,500	44,000	2,100*	21,000*	630	7,600
Soil	35	25,000	3,300,000	530	135,000	32	2,400
Root Vegetables	18	50,000	4,500,000	250	550,000	18	1,400
Leaf Vegetables	35	8,700	2,900,000	280	80,000	13	10,000

*1964 data only

initial marketing procedures on the farm were completed. The purpose was to demonstrate the effect of the market processing on coliform densities.

The confirmed, completed, and fecal coliform MPN's increased (Table II) from mean values of 250,000, 16,000, and 200 per 100 grams on crops taken from the field to 450,000, 76,000, and 1,300 respectively for the market root crops. The leaf vegetables showed some decreases in the mean values from field to market conditions.

TABLE II

Coliform Densities of Market and Field Vegetables Grown on Farms
Using Polluted Irrigation Water
Geometric Mean and 90th Percentile Distribution
of MPN Values per 100 Grams

	No.of	Confirmed Test		Completed Test		Fecal Test	
Vegetable	Samples	Mean	90th%	Mean	90th%	Mean	90th%
Root-Field	20	250,000	18,000,000	16,000	3,200,000	200	10,000
Root-Market	13	450,000	42,000,000	76,000	8,000,000	1,300	90,000
Leaf-Field	34	12,500	1,380,000	2,000	270,000	20	5,000
Leaf-Market	31	7,100	1,700,000	400	80,000	13	6,300

The water from a wash tank in which large quantities of root vegetables harvested from one growing area were routinely washed, was sampled and analyzed for coliform densities. In Table III are presented the mean values of both the source water entering the wash tank from a shallow well and the wash water in the tank after vegetables had been immersed, agitated, and removed. The source water from the inlet pipe was not of high bacteriological quality; the tank water was worse, containing over 35 times as many completed, and 3 times as many fecal coliforms.

TABLE III

Coliform Densities of Wash Water for Market Root Vegetables
Mean MPN Values

Sample	Number of Samples	Confirmed Test	Completed Test	Fecal Test
Source Water	7	29,000	11,000	2,000
Wash Tank Water	6	440,000	390,000	6,200

Table IV reports coliform levels representing the four maximum values found for vegetables in the survey. These results emphasized the very large numbers of completed and fecal coliforms which were detected on some vegetables.

TABLE IV

Four Maximum Coliform Densities from Single Vegetable Samples
MPN per 100 Grams

Vegetable	Type	Confirmed Test	Completed Test	Fecal Test
Radish	Field	1,300,000	490,000	79,000
Cabbage	Field	3,300,000	490,000	170,000
Radish	Market	2,300,000	1,300,000	1,300,000
Green Onion	Market	490,000,000	490,000,000	46,000

The geometric means and 90th percentile levels were obtained by plotting on log probability paper the log to the base ten of the coliform density of the samples. These graphs appear in Appendix B. They follow an approximate log normal distribution, particularly those representing larger numbers of samples. The geometric mean indicates the most probable value for coliforms per 100 milliliters of water or 100 grams of soil or vegetable. The 90th percentile level indicates that, on the average, 90 percent of the samples will have coliform levels of that magnitude or less.

Enteric pathogens were recovered from eight samples representing the South Platte River in the area where it was diverted into the irrigation ditches and from three of the polluted irrigation ditches. These organisms gave the typical biochemical and serological test reactions for Salmonellae. No Shigellae were recovered.

Salmonella organisms were found in one of five soil samples collected. This positive sample was taken one day after irrigation had taken place, from a field in which green onions were being grown. Enteric pathogens were not recovered from nine leaf and fifteen root vegetables tested.

Toxicity tests of the blended vegetable samples for coliform culture did not produce any positive results.

Appendix C contains bacterial densities, reported by individual farms, for irrigation water, soil, and vegetable samples. Appendix D lists chemical water quality data according to farm and source.

DISCUSSION OF RESULTS

It has been demonstrated (see Table I) that both the total (completed) coliform and fecal coliform group densities in the soils and on root vegetables are much higher in fields irrigated with highly polluted irrigation waters than in those irrigated with waters from relatively unpolluted sources. This was evidenced by the geometric mean densities which represent the central values for the data obtained and was underscored by the 90th percentiles which were close to the maximum values encountered during the investigation.

The contrast of total completed and fecal coliform counts from leaf vegetables irrigated with polluted versus clean water was not as great. Leaf vegetables had less direct contact with the irrigation water and the soils in which they were grown. They were also less protected from the environment-stabilizing effects of high temperatures, sunshine, and low humidity.

The 90th percentile total confirmed and fecal coliform results for leaf vegetables irrigated with comparatively clean water reflected high levels found on a few leaf lettuce samples from Farms 14, 15, and 16 (Appendix C), and were higher than their counterparts irrigated with polluted water.

The data from the Completed Test include all the members of the coliform group without regard for their native habitat or environmental origin. The fecal coliform test results are limited to that portion of the coliform group characteristic of the gut and fecal excreta.

The ratios between fecal coliform densities for water, soil, root vegetables, and leaf vegetables irrigated with polluted water and the comparable densities for those irrigated with relatively unpolluted water reveal the differences in results.

Ratios of Fecal Coliform Densities

Polluted Water/Relatively Unpolluted Water Used in Irrigation

	Mean	90th Percentile
Water	92.1	473.6
Soil	100	83.3
Root Vegetables	14.4	50
Leaf Vegetables	1.2	--

These data demonstrate the use of the more polluted irrigation water (average 92 times as polluted) resulted in 100 times the pollution in the soils and 14 times in root vegetables. There were no significant differences for leaf crops irrigated with polluted versus those irrigated with unpolluted water. At the 90th percentile level a similar pattern of decreasing values resulted.

The fecal portion of the coliform group used in these ratios represents microorganisms derived almost exclusively from animal or human excreta. They are organisms which may at any time be associated with the various diseases transmitted in the fecal excreta of warm-blooded animals (9). An increase of fecal coliform microorganisms results in a greater probability of other microorganisms of the fecal environment being present.

The geometric mean values for root and leaf crops taken from the field and those processed for market are listed in Table II. The fecal coliform mean density on field root crops was 200 as compared with 1300 for market processed root crops, an increase of 6-1/2 times. This increase in numbers of pollution indicator groups on root crops may point to the greater amount of handling and bunching of crops removed from contaminated soil and washing in poor quality water which takes place on some farms. In fact, the added moisture may favor more rapid bacterial growth. Comparison of fecal coliform data on the two groups of leaf vegetables indicates that there is no significant difference (20 and 13 respectively per 100 gm).

The use of a wash tank to rinse vegetables is often part of the preparation for marketing of root vegetables. The wash tank sampled was in use adjacent to one large growing area for vegetables sampled. The large concrete tank was constructed with an inlet pipe at one end and an outlet drain on the other. The confirmed, completed, and fecal coliform levels reported in Table III were much higher in the tank than in the source water. Neither water (2000 fecal coliforms per 100 ml of source water and 6,200 per 100 ml of water in the tank) demonstrated a desirable quality for rinsing vegetables to be consumed raw.

Table IV represents the four maximum values from vegetables during the two-year investigation. These four counts were excessively high for foods which are normally eaten without cooking, regardless of the interpretation which may be used in the evaluation of coliform data.

In summation of the evaluation and interpretation of the data tabulated in Tables I, II, and IV, it has been demonstrated that total coliform group densities increased as the quality of the irrigation waters decreased. Fecal microorganisms in various concentrations found their way from the irrigation water to root crops and leaf vegetables. Investigators over the years have established that as the bacteria characteristic of fecal excreta increase, the relative probability of hazard from enteric diseases increases. Factual evidence has been presented demonstrating the presence of fecal coliform bacteria and their relative densities on vegetables irrigated with both polluted and relatively clean waters.

It is not within the scope of this investigation to establish recommended criteria for irrigation waters, nor for vegetables consumed raw or cooked. These data, with their interpretations, should, however,

provide facts and evidence for guidance to those who have the responsibility for making such decisions and the establishment of an enforcement program. The geometric mean values represent the nearest approach to the most probable pollution levels for the vegetables under study. Those values of the 90th percentile and higher represent maximum values found and therefore must be taken into account in determining the relative hazard. Proof of actual transmission of disease without comparative bacteriological and epidemiological data is not possible from this investigation. Conversely, a careful analysis of the data with the proof of the presence of fecal coliform bacteria on vegetables consumed in the raw state, definitely does not exclude the absence of a health hazard due to pathogenic enteric entities.

The polluted irrigation waters sampled during the 1964 season and analyzed chemically produced median dissolved oxygen values ranging from 0.0 to 2.3 milligrams per liter and median biochemical oxygen demands ranging from 24 to 63, whereas the comparatively clean waters resulted in ranges of 8.2 to 9.4 and 1.0 to 2.4 respectively (Appendix D).

Sanitary field inspections during the growing season of the areas utilizing grossly polluted water for truck crop irrigation revealed an unsightly appearance and the pungent odors typical of putrifying waste materials. The water in the ditches foamed below outfalls and at headgates due to the presence of sewage associated detergents, and carried many visible solids including feces, disintegrated toilet paper, and contraceptive devices. A thick layer of sludge was observed on the bottom and along the sides of some ditches. Some of these solids were found deposited on the soil in the furrows of the vegetable plots. Sewage, pooled irrigation water, and sludge banks along the ditches attracted and provided breeding places for insect vectors, flies, and mosquitoes, which in turn are also carriers of enteric and other diseases (10).

SUMMARY OF OTHER INVESTIGATIONS

Survival of Enteric Organisms

The survival of pathogens in water and soil is dependent upon environmental and physiological factors. Some of the significant factors in the environment are: pH, temperature, moisture, sunlight, available oxygen, toxic materials, nutrient supply, population density, and the antibiotic or competitive activities of other organisms. Important physiological conditions are the ability of the organisms to form resistant spores or cysts; their reaction to heat, cold, chemicals, and killing agents; and their ability to compete with other organisms. In order for pathogens to cause disease, they must be virulent, present in sufficient numbers, and able to gain entry into a susceptible host.

There have been conflicting opinions on the relative survival of coliform and pathogenic bacteria in water and soil and on vegetation irrigated or fertilized with fecal contaminated materials. Rudolfs et al reviewed the literature (11) and indicated that fruits and vegetables growing in polluted soil can become contaminated with pathogenic organisms; that these bacteria may survive for periods of a few days to several weeks in the soil and on vegetation and that infected vegetation may be difficult to cleanse and disinfect, particularly if the fruits or vegetables have abrasions or broken skins. Falk (12) and Rudolfs, Falk and Ragotzskie (13) found coliform bacteria detectable on the surfaces of growing crops one month after application; whereas Salmonella and Shigella species disappeared within seven days. Mallman and Litsky (14) observed coliforms persisting in soil for longer periods than either typhoid bacteria or fecal streptococci. Clark (15) recovered pathogenic bacteria from water and sewage after several days in warm weather, and after several weeks in cold weather. Harvey reported the prolonged survival of Salmonella paratyphi B in sewers (16). Allen and Smith (17) believed that coliforms are more resistant to sewage treatment processes and were demonstrable for a longer period of time than fecal streptococci. Baars (18) found E. coli persisting in ground water two to three years after contamination by seepage from pit privies.

Salmonellae have been isolated from the Red River of the North (19) as far as 73 miles and 4 days' time of travel downstream of sewage outfalls after a 95-99 percent reduction in mean coliform densities. Laboratory survival studies (20) have indicated that the rates of growth and dieoff for Salmonella typhimurium and fecal coliforms are different under varying environmental conditions. Large reductions in fecal coliform concentrations may not necessarily insure comparative reductions of Salmonellae.

Sewage Irrigation Practices in Western United States

The public health hazards associated with the use of sewage-contaminated water for irrigation have been recognized in both the United States and Europe in regions which have followed these practices for decades. Standards have been formulated for the protection of the public from the transmission of enteric diseases caused by intestinal pathogenic bacteria, viruses, parasitic protozoa, and worms. However, such standards vary greatly and are not widely practiced.

The primary purpose of sewage irrigation in the Western United States is water reclamation; fertilization is a secondary factor. Sewage irrigation regulations in the western states generally have prohibited the use of raw or settled sewage on vegetables grown for human consumption. However, some states (21) allow the use of completely treated and reliably disinfected sewage on fruits and vegetables to be eaten raw and for forage crops. Others prohibit the use of any sewage effluent for these purposes. In some cases irrigation with settled or partially treated sewage is specified only for field crops such as grains, fodder crops, and some vegetables which generally are never consumed uncooked. Chlorination of the effluent has been requested by some State health agencies for the protection of field workers. In order to aid enforcement, some states adopted a scheme of licensing and permits which specify the type of crops to be raised, quality of irrigation water, and growth period during which water may be applied (21).

In a thorough review of irrigation practices in the Western States (22) Wright mentioned the possible danger to field laborers, food handlers, and the ultimate consumer from handling and consuming fruits and vegetables irrigated with polluted waters. It was pointed out that irrigation of vegetables with polluted water is objectionable to health authorities as well as the ultimate consumer from the standpoint of aesthetics. Such considerations are important because they may affect the market for these vegetables. He concluded that, "In the absence of widely accepted requirements or standards, health authorities are faced with the necessity of requiring a high degree of treatment for domestic and industrial wastes where irrigation waters are involved in order to eliminate possible health hazards."

Three sets of State regulations and objectives, one long-standing and the other two recently adopted, illustrate current thinking in regard to criteria for and control of irrigation practices. The California State Department of Health (23) adopted in 1933 rules governing the use of sewage for crop irrigation purposes which prohibit: "(1) Raw or untreated sewage containing human excrement for irrigating growing crops; (2) Undigested or untreated sludge for fertilizing any growing vegetables, garden truck, low-growing fruits or berries; (3) Settled or undisinfected sewage effluents to be used to water any

growing vegetables, garden truck, berries, low-growing fruits or vineyards during seasons in which windfalls or fruit lie on the ground; nursery stock, vegetables grown for seed, cotton, sugar beets, grains, fodder crops may be watered with such undisinfected or partially disinfected sewage effluents provided that no milch cows are pastured on the land while it is moist with sewage; (4) Highly treated sewage to be used for the irrigation of edible crops must meet the bacteriological requirements of the U. S. Public Health Service Drinking Water Standards, i.e., less than one coliform per 100 ml."

The Missouri Basin Health Council in 1954 adopted standards for the Missouri Drainage Basin (23). The Missouri Water Pollution Board has adopted these as objectives for the Missouri, Mississippi, and Blue Rivers. Nebraska's Water Pollution Control Council adopted in 1962 these same standards as objectives for the bacteriological quality of irrigation water and specified: "(1) Sewage used for irrigation of forage crops should be given treatment insuring a consistent reduction in coliform bacteria of not less than 90 percent; (2) Sewage used for irrigation of human food crops should be given treatment insuring a consistent reduction in suspended solids of not less than 75 percent, in five-day 20°C BOD of not less than 75 percent and in coliform bacteria of not less than 98 percent, unless discharged into a stream where the volume of dilution water available during the low flow periods, during which irrigation diversions may occur, is at least ten times greater than the anticipated maximum daily volume of sewage, in which case a bacterial reduction of 90 percent may be acceptable; provided, however, that where the time of passage from plant discharge to irrigation diversion is such as to permit the normal death rate of bacteria as evidenced by the die-away curve to provide equivalent bacterial reduction, the requirements of (1) and (2) may be appropriately modified."

The State of Utah set bacteriological limits for irrigation waters in its "Standards of Quality and the Regulations for Water Classification" (24) in 1954, amended in 1960. These standards state that no person shall discharge into Class "C" (irrigation and other specified uses) waters any wastes which result in a monthly arithmetical average MPN of coliform organisms exceeding 5,000 per 100 ml; or in an MPN exceeding this number in more than 20 percent of the samples collected during any month; or in an MPN exceeding 20,000 per 100 ml in more than 5 percent of such samples, or which result in a monthly arithmetical average BOD in said waters exceeding 5 ppm; or in a BOD exceeding this amount in more than 20 percent of the samples collected in any month; or in a BOD exceeding 10 ppm in more than 5 percent of such samples. Class "D" waters are to be suitable for general irrigation but not for irrigating lawns, recreational areas, pastures used for dairy cattle, root crops, or any low-growing crops produced for human consumption. The State designates that the

bacterial standards are the same as for Class "C" waters, but the BOD levels are more relaxed--a monthly average of 25 ppm not to be exceeded by 20 percent, and 50 ppm not to be exceeded by 5 percent of the samples. There are no regulations governing sewage irrigation in Colorado.

Sewage Irrigation Practices in Europe

In Europe, the fertilizing value of sewage is an economic necessity as much as the need for the water itself. Although sewage irrigation is generally considered hygienically safe, some areas stipulate (21) that sewage-polluted water should not be used on truck crops or vegetables. Many European communities (25) insist upon biological treatment before use for irrigation; others maintain (26) that secondary treatment of sewage for agricultural use is economically unfeasible. Use of settled sewage is prohibited by some and allowed by others under certain conditions, including furrow irrigation of tomatoes, carrots, and radishes (27).

European regulations (21) generally require a minimum time interval be established between cessation of sewage irrigation and harvesting of vegetables and forage crops. This period of time varies from "before planting" to "one week before harvesting."

The use of highly polluted water is not generally permitted close to water treatment or storage facilities or dwellings (28) (29). Adequate safeguards must prevent the contamination of surface or ground water. Workers (29) are vaccinated against intestinal diseases, inspected for worm infection, and instructed in personal hygiene when working with polluted water.

One observed European standard (30) for irrigating truck crops is that the water conform to open water swimming standards. Certain types of wastes, such as those from industries using raw animal products (e.g. slaughterhouses and tanneries), and hospitals are prohibited from such irrigation use (29) (31). Sludge used as fertilizer must be dried and composted.

Spray irrigation (21) (27) (29) (30) is considered more hazardous than furrow irrigation and is often forbidden on fruits and vegetables along with other restrictions.

Some European communities (21) (27) (29) (31) point out that harvested vegetables should not be laid on the bare ground and fallen fruit should not be eaten raw. It is recommended that fruit and vegetables shall be hosed with pure running water before being taken to market.

Previous Colorado Studies

The serious contamination of the South Platte River by untreated Denver sewage and the disproportionately large number of enteric fever cases in the Platte Valley below Denver were pointed out by Hall (32) in 1933.

The high incidence of infectious intestinal diseases in Colorado and the need for sewage treatment in areas receiving polluted irrigation water in Western States were stressed by Chapman (33) (34) (35) in 1934-35. He noted that the Colorado State Board of Health had found a large number of coliform bacteria on vegetables raised with sewage-contaminated irrigation water many miles below the source of pollution. Chapman emphasized that a real danger existed when contaminated soils and waters contact market produce consumed in the raw state. He further suggested that handling or storage in the refrigerator may transmit pathogenic bacteria to other foods eaten raw. The possible hazard to field workers, market laborers, and housewives handling these materials was stressed.

In 1946 Gaub (36) reported that 250 men had contracted enteritis and diarrhea in a military installation near Denver. Raw cabbage served as cole slaw was the suspected agent and the shipment of cabbage was traced to truck farms north of Denver utilizing sewage-laden irrigation water. Shigella dysenteriae was isolated from another shipment of cabbage taken from the same fields. However, the source of contamination was inconclusive because of the time interval between the outbreak and the field investigations.

A sanitary survey of the South Platte River Basin in 1950 (37) indicated that the irrigation waters contained high coliform densities by the confirmed test procedure and high enterococcus levels by the relatively inhibitory Winter-Sandholtzer method, together with a large number of Salmonella organisms. The coliform levels of leafy vegetables were higher than those of smooth-skinned vegetables. Norman and Kabler in a discussion of these data (38) further observed that the coliform counts on vegetables irrigated with waters of high coliform density were higher than those irrigated with relatively pure water. Coliform levels of the soil reflected those of the irrigation water they received.

Dunlop et al (39) (40) (41) recovered Salmonellae and other pathogenic organisms from a significant number of irrigation water samples contaminated with effluent from the Denver Northside primary sewage treatment plant from 1951 through 1961. Similar organisms were found once on celery irrigated with this water. Dunlop stated, in part, (42) "It would appear that the public health hazard associated with the use of sewage-contaminated water for the irrigation of truck crops to be consumed raw is perhaps not so great as has been

assumed in the past. Nevertheless, the fact that the water has been shown to be contaminated indicates that a potential hazard still exists, and that every effort must be made to provide adequate treatment of all domestic and industrial wastes before these wastes are discharged into streams to be used later for irrigation purposes."

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APPENDICES

APPENDIX A

Agriculture, Irrigation, and Sampling Information by Farm and Source

Agricultural, Irrigation, and Sampling Information
by Farm and Source

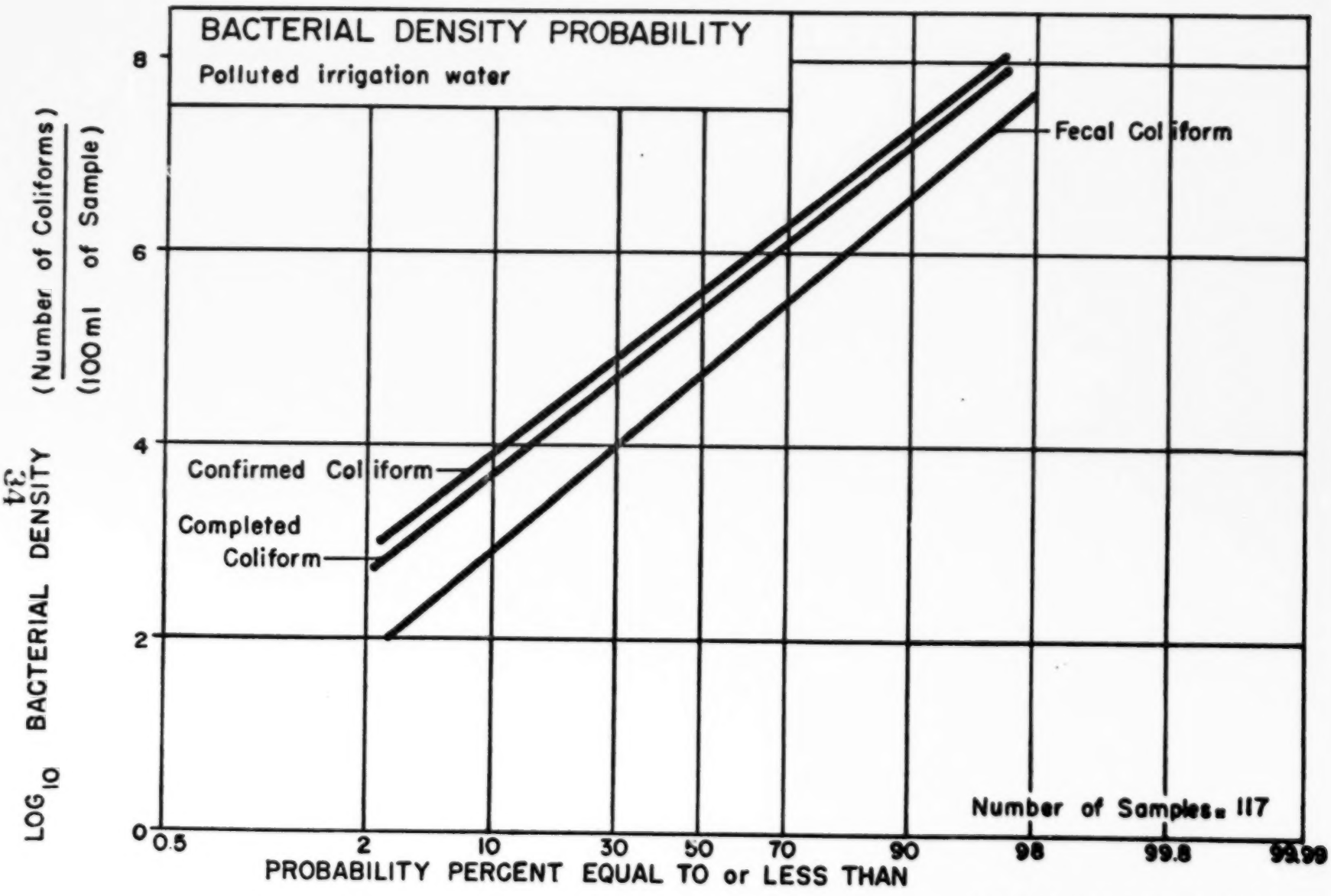
Farm No.	Vegetables Sampled	Period Sampled	Source of Irrigation Water	Irrigation Method	Fertilizer Used	Remarks
1	Radishes, cabbage, peppers, beets, onions	8/29/63 to 9/12/63	Seepage from Hodgson and Warrior Ditches, plus domestic water	Furrow and spray	Commercial. No manure used.	--
2	Lettuce, parsley, radishes, cabbage, collard greens, mustard greens	5/26/64	Headgate directly on Burlington Ditch only source of irrigation water	Furrow only	Commercial only	--
3	Radishes	9/2/63 to 9/12/63	Gardeners Ditch only source of irrigation water	Furrow only	Commercial only	--
4	Radishes, cabbage, green onions	8/29/63 to 9/12/63	Gardeners Ditch only source of irrigation water	Furrow only	Commercial only	--
5	Cauliflower, head lettuce, cabbage	8/29/63-9/13/63;and 5/25/64-9/1/64	Gardeners Ditch only source of irrigation water	Furrow only	Commercial only	--
6	Lettuce, radishes, green onions, parsley, endive	5/25/64-9/14/64	Gardeners Ditch only source of irrigation water	Furrow only	Commercial only	--
7	Peppers, cabbage, onions, leaf lettuce, romaine lettuce, head lettuce	8/29/63-9/12/63;and 5/25/64-7/20/64	Duggan Ditch, also some well water	Furrow only	Commercial only	Ditch rights to Duggan Ditch sold effective 8/1/64

Farm No.	Vegetables Sampled	Period Sampled	Source of Irrigation Water	Irrigation Method	Fertilizer Used	Remarks
8	Radishes, parlsey	5/25/64-7/20/64	Duggan Ditch plus well water	Furrow only	Commercial	Sold water rights to Duggan Ditch effective 8/1/64
9	Carrots	5/26/64	Burlington Ditch via 1-5 miles of feeder canal plus well water	Furrow	Commercial	Only well water applied one month prior to harvest
10	Celery	5/26/64-9/28/64	Fulton Ditch plus well water	Furrow	Commercial, and manure in late fall	Ceased application of ditch water one month prior to harvest
11	Turnips, leaf lettuce, onions	8/29/63-9/12/63	Brantner Ditch only source of irrigation water	Furrow	Commercial only	--
12	Turnips, cabbage	8/29/63-9/12/63;and 5/26/64-9/29/64	Fulton Branch only source of irrigation water	Furrow	Commercial only	--
13	Cabbage	8/29/63-9/12/64	Fulton Branch only source of irrigation water	Furrow	Commercial only	--
14	Leaf lettuce, carrots	7/1/64-8/12/64	Left Hand Ditch; also well water	Spray	Manure in late fall	

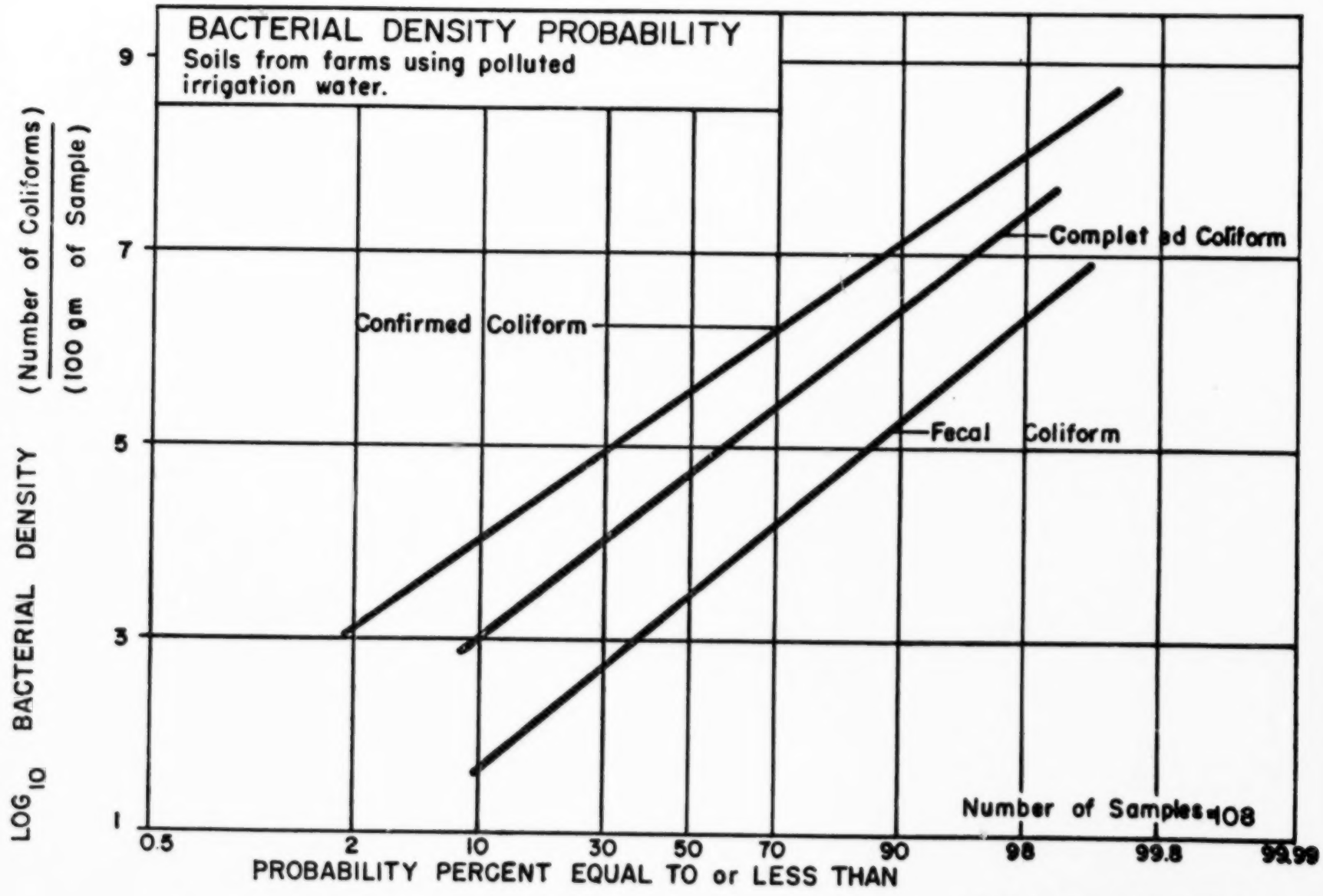
Farm No.	Vegetables Sampled	Period Sampled	Source of Irrigation Water	Irrigation Method	Fertilizer Used	Remarks
15	Leaf lettuce, carrots	7/15/64-8/5/64	Left Hand Ditch; also well water	Spray	Manure in late fall	--
16	Leaf lettuce, carrots	6/3/64-9/2/64	Left Hand Ditch; also well water	Spray	Manure in late fall	--
17	Cabbage	5/28/64-9/16/64	Niwot Ditch only source of irrigation water	Furrow	Commercial only	--
18	Leaf lettuce, radishes, carrots	5/28/64-9/2/64	Swede Ditch; also well water	Spray	Manure in late fall	--
19	Carrots	6/3/64-9/28/64	Rough and Ready Ditch only irrigation water used	Furrow and Spray	Commercial, plus manure in fall	--

APPENDIX B

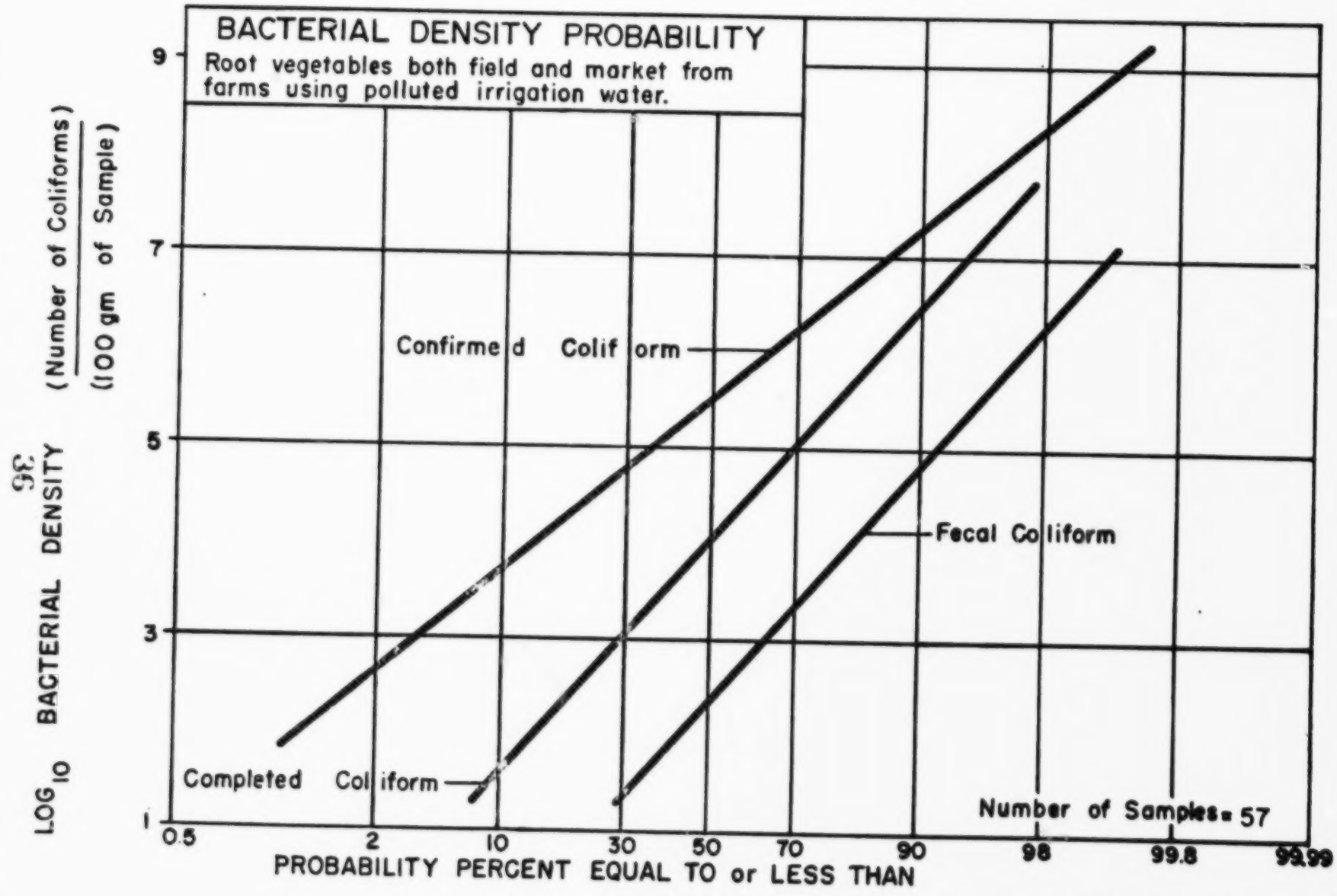
Bacterial Density Probability Graphs



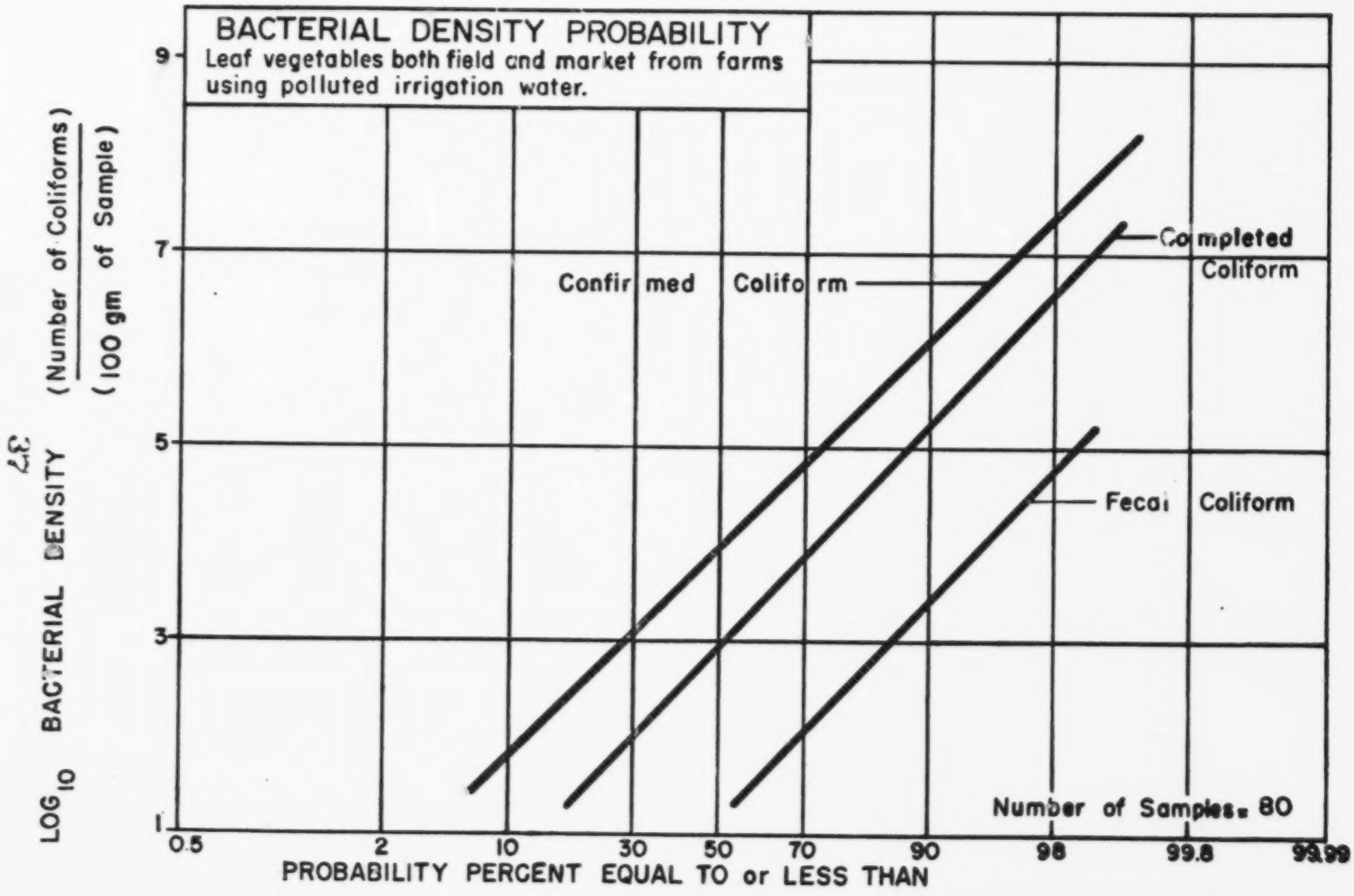
APPENDIX B - FIGURE 1



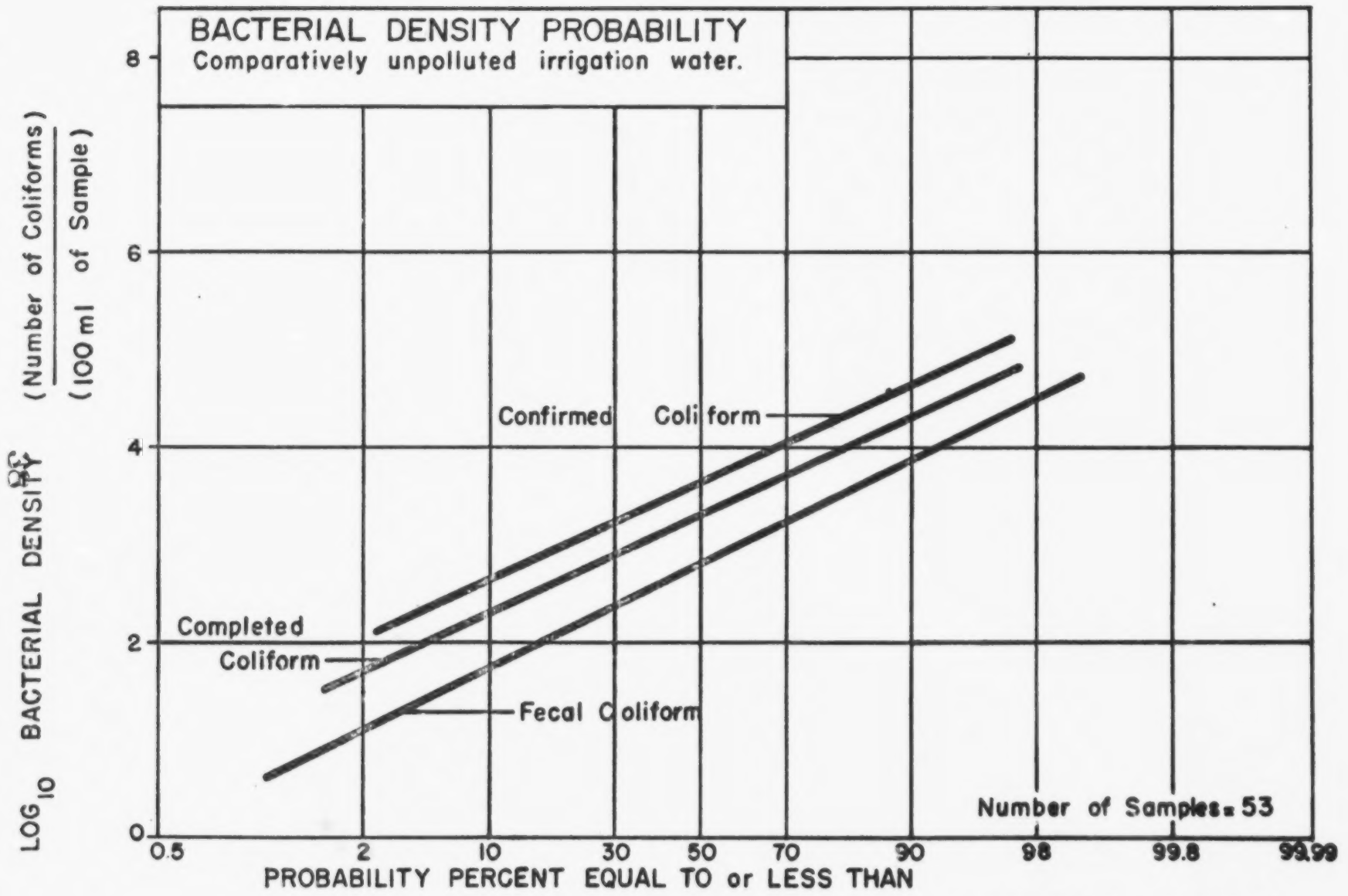
APPENDIX B - FIGURE 2



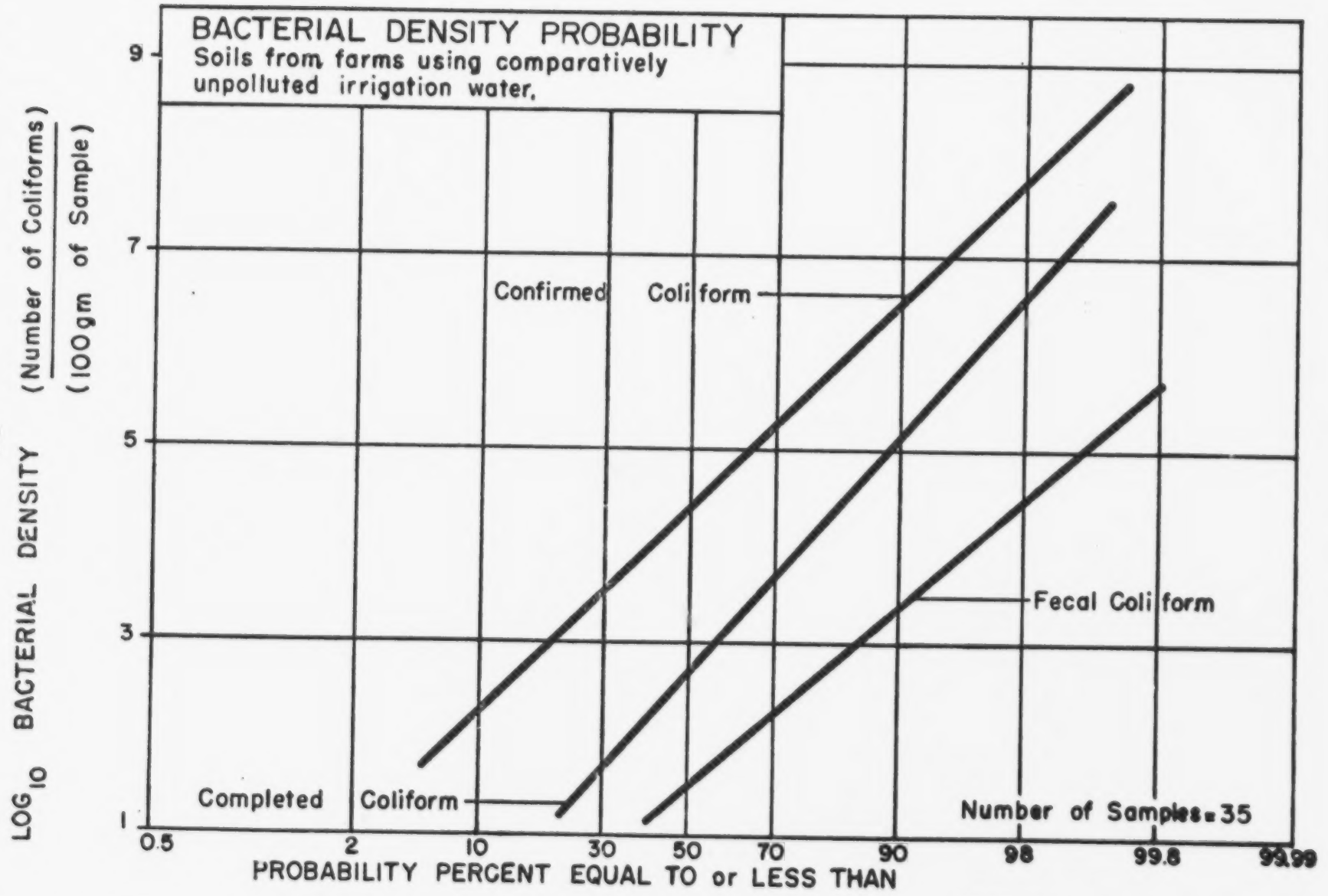
APPENDIX B - FIGURE 3



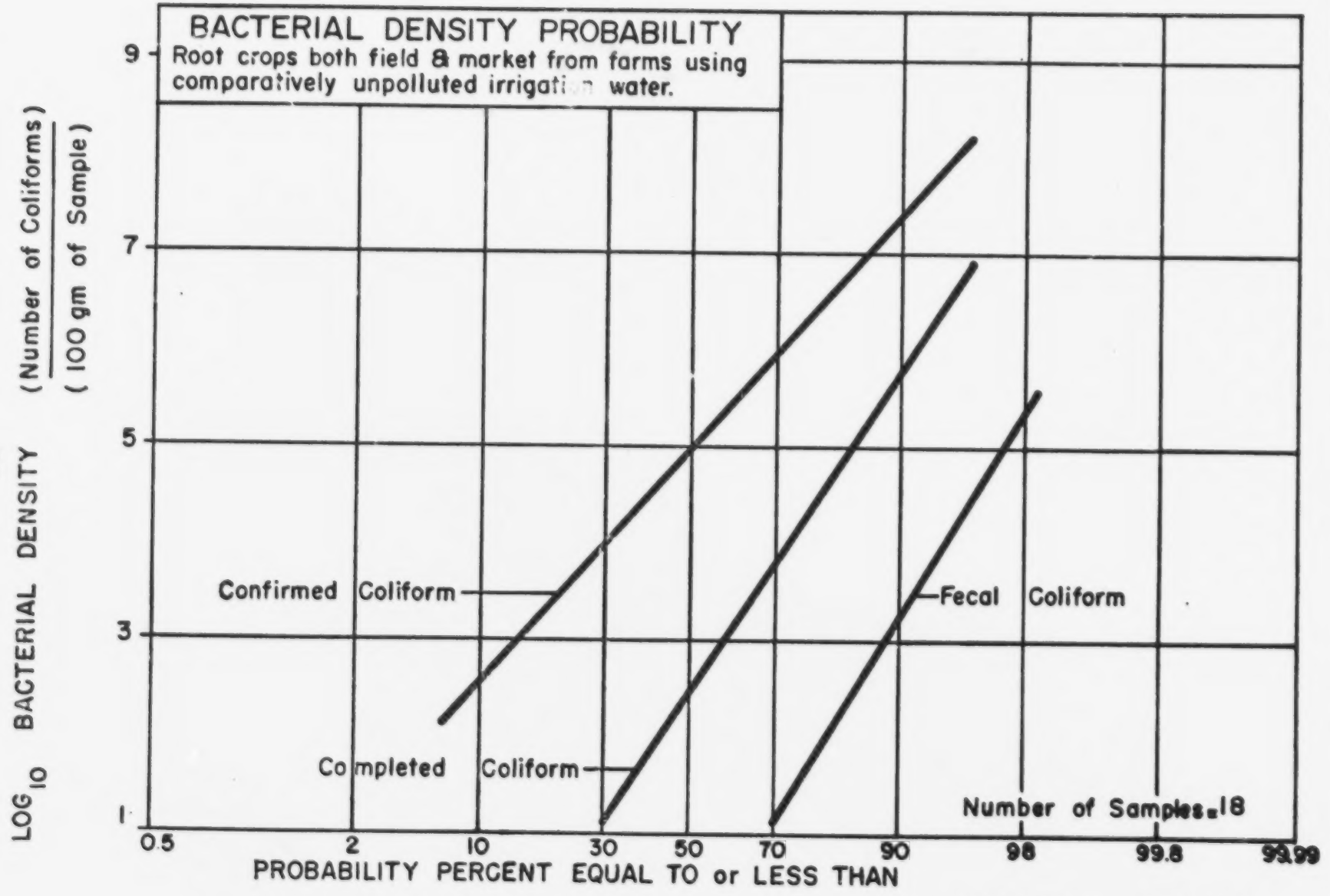
APPENDIX B - FIGURE 4



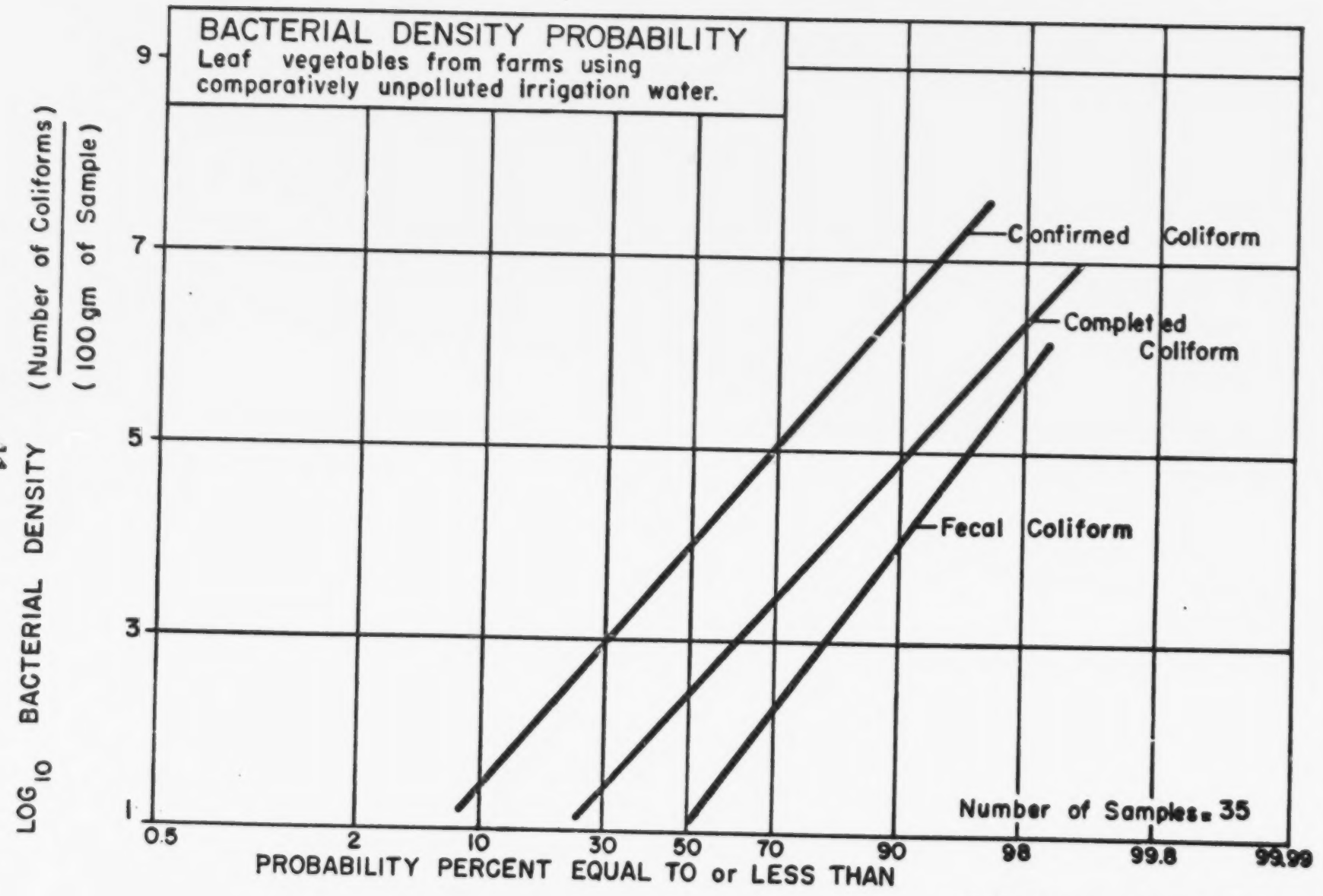
APPENDIX B - FIGURE 5



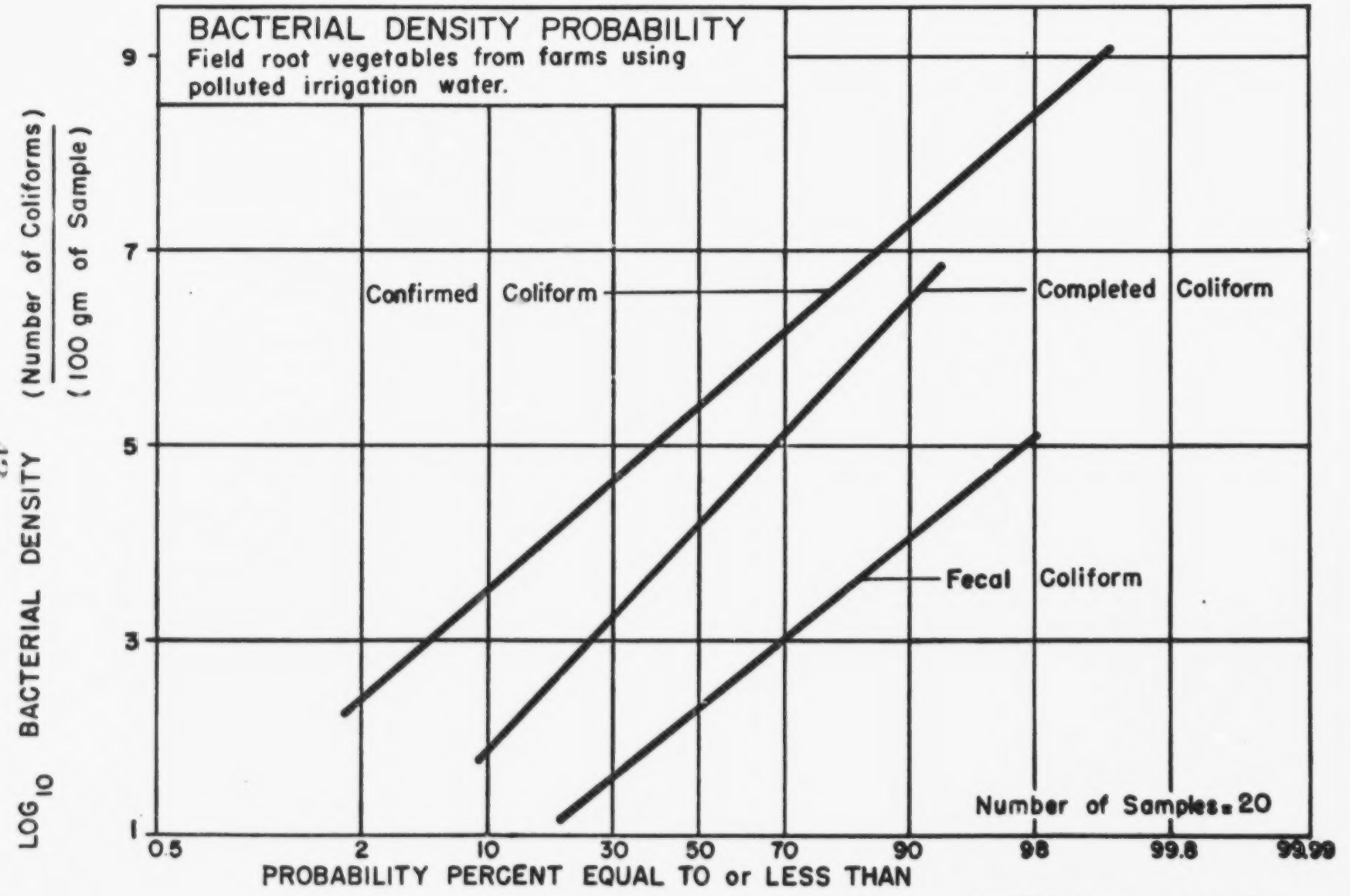
APPENDIX B - FIGURE 6



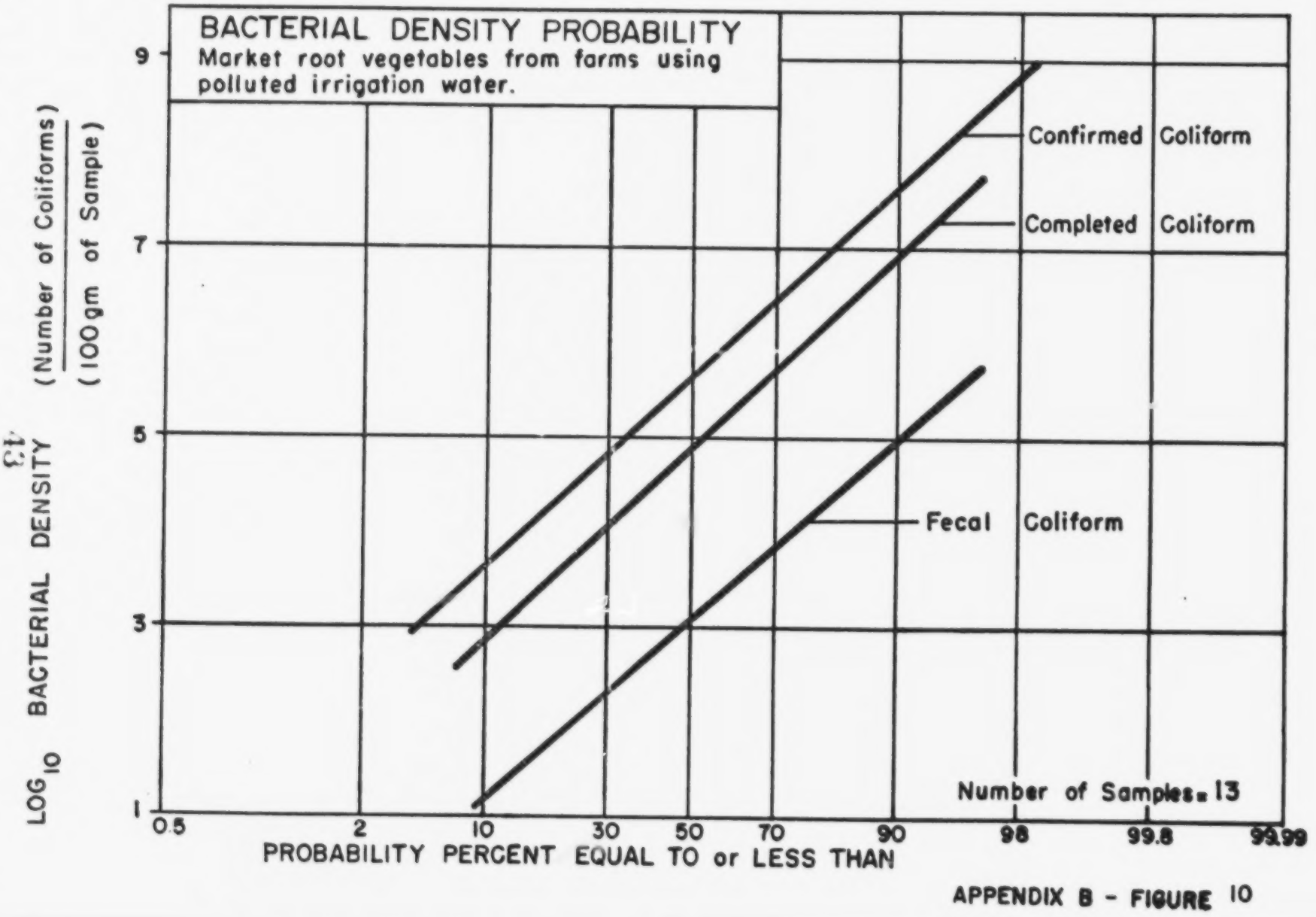
APPENDIX B - FIGURE 7



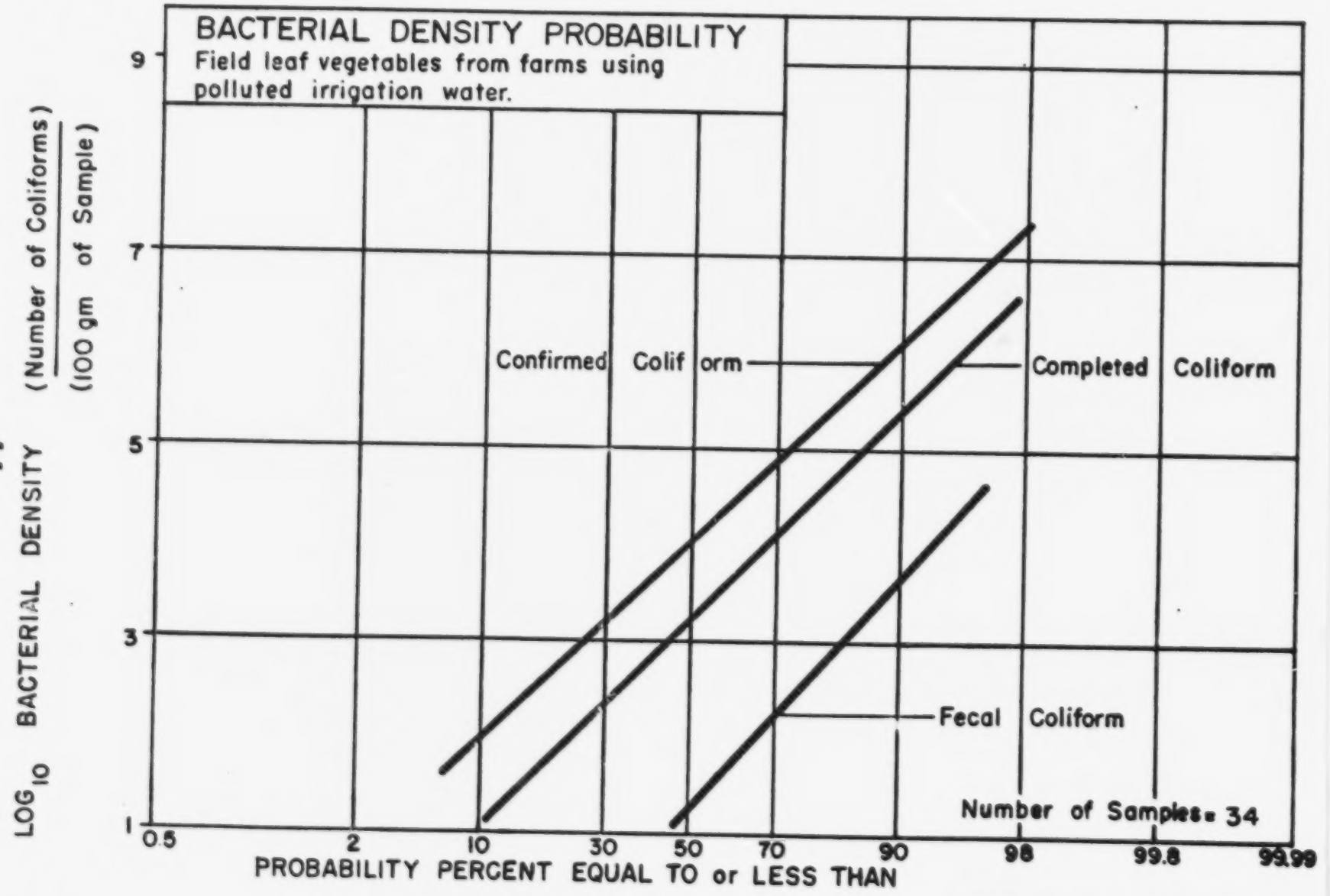
APPENDIX B - FIGURE 8



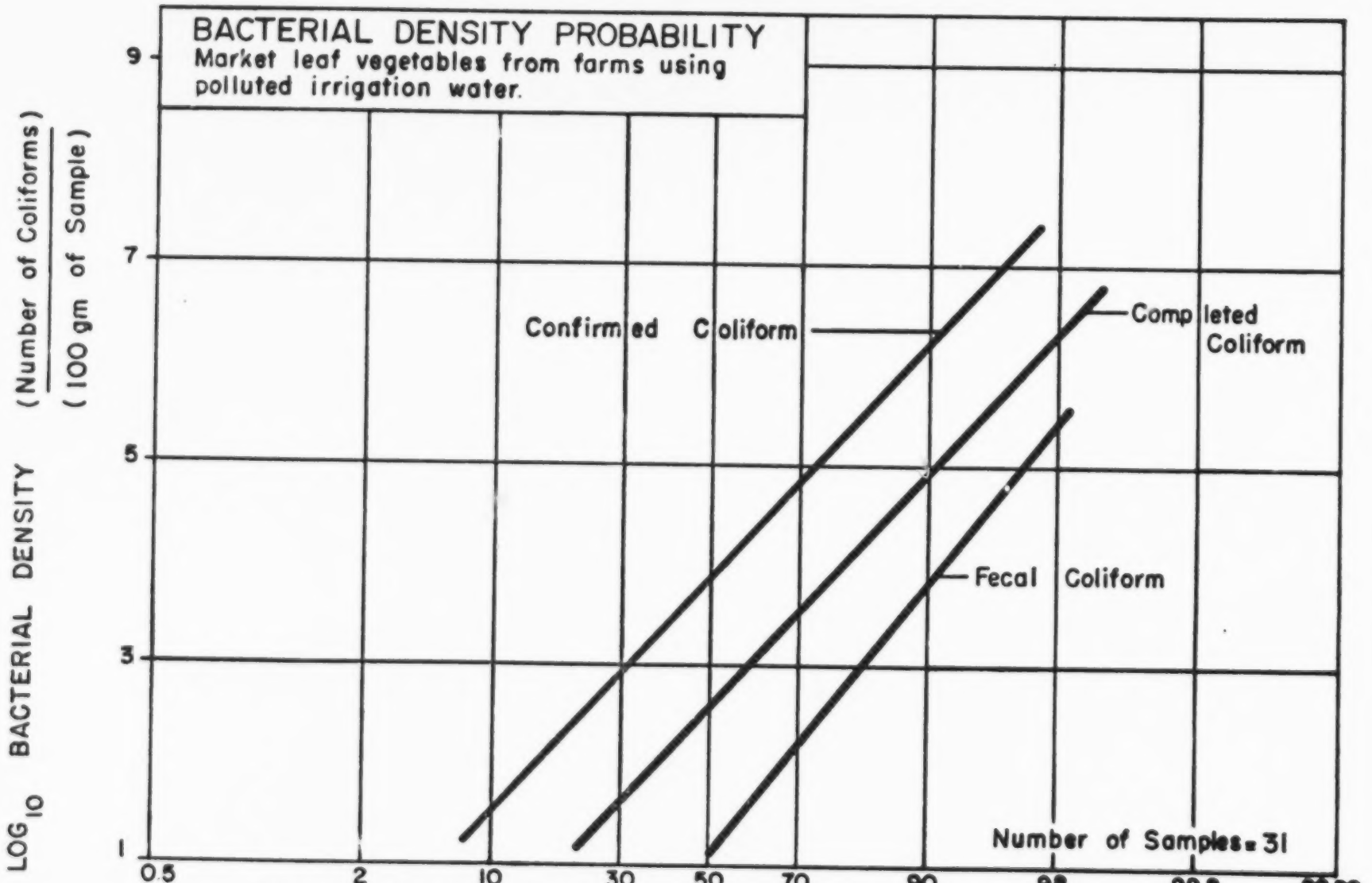
APPENDIX B - FIGURE 9



APPENDIX B - FIGURE 10



APPENDIX B - FIGURE II



APPENDIX B - FIGURE 12

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER
ACCORDING TO FARM AND SOURCE
MPN/100 ml or /100 gram of sample

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 1 - 1963 Study. Irrigation Source: Seepage from Hodgson and Warrior Ditch										
Vegetables:										
Onion	4	4,600	>160,000	8,400	< 20	< 20	< 20	< 20	< 20	< 20
Radish	3	1,000	$> 1.6 \times 10^6$	1.6×10^6	20	17,000	8,500	< 20	< 20	< 20
Cabbage	3	60	280,000	17,000	< 20	220	120	< 20	< 20	< 20
Beets	3	500	540,000	49,000	40	22,000	70	< 20	< 20	< 20
Soil:	7	2,200	1.6×10^6	8,400	< 20	4,600	20	< 20	< 40	< 20
Irrigation Water:	17	280	92,000	3,300	--	--	--	8	13,000	490
Farm No. 2 - 1964 Study. Irrigation Source: Burlington Ditch										
Vegetables:										
Cabbage - Field	4	45	49,000	7,200	18	33,000	200	< 18	20	< 18
Cabbage - Market	2	700	4,900	2,800	18	170	80	< 18	< 18	< 18
Chinese Cabbage-Field	1	--	--	46,000	--	--	4,900	--	--	460
Chinese Cabbage-Market	1	--	--	330	--	--	18	--	--	18

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 2 - 1964 Study (cont'd)										
Vegetables:										
Leaf Lettuce - Field	2	23,000	1.7×10^6	860,000	4,400	23,000	14,000	< 18	23,000	12,000
Parsley - Market	6	790	1.3×10^6	19,100	78	140,000	430	20	17,000	33
Parlsey - Field	4	1,700	95,000	25,500	< 18	95,000	9,000	< 18	790	130
Radishes - Field	3	1.3×10^6	1.7×10^6	1.6×10^6	28,000	1.7×10^6	1.6×10^6	61	14,000	13,000
Collard Greens - Market	4	170	7.0×10^6	1.25×10^6	< 18	1.3×10^6	250,000	< 18	7,000	690
Mustard Greens - Market	4	40	110×10^6	110,000	< 18	7.0×10^6	1,700	< 18	2.2×10^6	1,100
Soil:	13	33,000	54×10^6	490,000	11,000	13×10^6	140,000	< 18	170,000	4,900
Irrigation Water:	33	110	70×10^6	110,000	27	35×10^6	49,000	2	13×10^6	13,000
Farm No. 3 - 1963 Study. Irrigation Source: Gardeners Ditch										
Vegetables: Radishes	3	11,000	59,000	28,000	130	7,900	330	< 20	70	70
Soil:	3	4,900	350,000	13,000	120	700	140	< 20	50	20
Irrigation Water:	23	170,000	3.48×10^6	790,000	--	--	--	17,000	2.2×10^6	170,000

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 4 - 1963 Study. Irrigation Source: Gardeners Ditch										
Vegetables:										
Radishes	1	--	--	12,000	--	--	--	--	--	80
Cabbage	2	49,000	920,000	484,500	23,000	49,000	36,000	< 20	< 20	< 20
Pepper	2	< 20	330	180	< 20	20	20	< 20	< 20	< 20
Onions	2	33,000	1.6×10^6	816,500	50	3,500	1,800	20	220	120
Soil:	6	1,300	23×10^6	20,000	50	1,300	150	20	1,300	40
Irrigation Water:	23	170,000	3.48×10^6	790,000	--	--	--	17,000	2.2×10^6	170,000
Farm No. 5 - 1963 Study. Irrigation Source: Gardeners Ditch										
Vegetables:										
Cauliflower	3	< 20	7,900	700	< 20	90	55	< 20	< 20	< 20
Soil:	3	79,000	920,000	350,000	140	172,000	86,700	< 20	790	220
Irrigation Water:	23	170,000	3.48×10^6	790,000	--	--	--	17,000	2.2×10^6	170,000
Farm No. 5 - 1964 Study. Irrigation Source: Gardeners Ditch										
Vegetables:										
Head Lettuce	1	--	--	3,300	--	--	340	--	--	< 18
Cabbage - Market	2	950	170,000	85,000	45	17,000	8,530	< 18	< 18	< 18
Cauliflower - Market	2	< 18	49,000	24,500	< 18	17,000	8,500	< 18	490	260

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 5 - 1964 Study (cont'd)										
Soil:	8	3,500	14×10^6	1.77×10^6	310	3.3×10^6	300,000	< 18	79,000	37,300
Irrigation Water:	17	17,000	3.3×10^6	490,000	11,000	2.3×10^6	330,000	1,100	1.7×10^6	4,900
Farm No. 6 - 1964 Study. Irrigation Source: Gardeners Ditch										
Vegetables:										
Leaf Lettuce	3	20	1.4×10^6	2,200	< 18	130,000	170	< 18	7,000	< 18
Radishes - Field	6	7,000	1.6×10^6	33,500	330	> 140,000	5,800	< 18	490	220
Radishes - Market	3	720,000	2.3×10^6	1.4×10^6	40,000	1.3×10^6	490,000	1,300	1.3×10^6	4,600
Green Onions - Field	5	490	16×10^6	230,000	13,000	1.7×10^6	490	< 18	220	< 18
Green Onions - Market	8	6,400	490×10^6	230,000	330	490×10^6	110,000	< 18	46,000	510
Parsley - Field	2	< 18	11,000	5,500	18	790	400	< 18	230	130
Endive - Field	3	430,000	4.9×10^6	490,000	25,000	1.7×10^6	330,000	45	23,000	7,000
Endive - Market	2	3,300	1.8×10^6	902,000	330	12,000	6,200	45	23,000	1,200
Soil:	24	11,000	5.4×10^9	945,000	3,500	19×10^6	220,000	330	230,000	3,200
Irrigation Water:	18	17,000	3.3×10^6	490,000	11,000	2.3×10^6	330,000	1,100	1.7×10^6	49,000

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 7 - 1963 Study. Irrigation Source: Duggan Ditch										
Vegetables:										
Peppers	2	< 20	70	50	--	--	20	< 20	< 20	< 20
Cabbage	2	79,000	79,000	79,000	--	--	20	< 20	200	110
Onions	4	920,000	$>1.6\times10^6$	1.26×10^6	--	--	1.6×10^6	< 20	240,000	12,500
Soil:	4	490	1.3×10^6	39,000	--	--	13,000	200	33,000	700
Irrigation Water:	9	79,000	3.3×10^6	790,000	--	--	--	23,000	1.09×10^6	330,000
Farm No. 7 - 1964 Study										
Vegetables:										
Leaf Lettuce - Field	3	700	17,000	1,700	260	4,900	1,700	18	490	330
Head Lettuce - Field	3	490	790,000	23,000	220	280,000	23,000	18	18	18
Romaine Lettuce - Field	4	490	240,000	3,000	68	9,500	700	18	1,100	18
Soil:	8	7,900	9.5×10^6	280,000	2,800	1.1×10^6	1.17×10^6	180	79,000	5,100
Irrigation Water	8	7,000	1.3×10^6	130,000	4,900	1.3×10^6	64,000	270	130,000	27,000

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATERS

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 8 - 1964 Study. Irrigation Source: Duggan Ditch										
Vegetables:										
Radishes - Field	6	3,500	3.3×10^6	1.4×10^6	360	490,000	97,000	170	79,000	400
Parsley - Field	2	< 18	49,000	24,500	< 18	17,000	8,500	< 18	790	400
Soil:	8	7,900	7.9×10^6	140,000	450	790,000	90,000	61	170,000	5,600
Irrigation Water:	8	23,000	460,000	170,000	7,900	220,000	140,000	490	79,000	28,000
Farm No. 9 - 1964 Study. Irrigation Source: Burlington Ditch										
Vegetables:										
Carrots - Market	2	2,800	330,000	166,000	1,100	22,000	11,500	< 18	7,900	3,960
Soil:	6	1,300	22×10^6	895,000	470	1.3×10^6	86,500	< 18	49,000	1,780
Irrigation Water:	11	79,000	33×10^6	1.1×10^6	22,000	33×10^6	700,000	1,700	7.9×10^6	310,000
Farm No. 10 - 1964 Study. Irrigation Source: Fulton Ditch										
Vegetables:										
Celery - Field	2	230,000	1.4×10^6	820,000	79,000	1.4×10^6	740,000	< 18	< 18	< 18
Celery - Market	4	< 18	35,000	24,600	< 18	2,200	50	< 18	< 18	< 18
Soil:	7	33,000	17×10^6	170,000	2,800	2.8×10^6	43,000	1,300	330,000	9,500
Irrigation Water:	9	330,000	79×10^6	1.7×10^6	330,000	79×10^6	1.7×10^6	46,000	11×10^6	490,000

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 11 - 1963 Study. Irrigation Source: Brantner Ditch										
Vegetables:										
Turnips	3	27,800	$>1.6\times10^6$	542,000	70	920,000	7,900	20	230	50
Leaf Lettuce	3	700	46,000	4,600	20	2,300	1,100	20	20	20
Onions	1	--	--	$>1.6\times10^6$	--	--	$>1.6\times10^6$	--	--	920,000
Soil:	5	17,000	1.6×10^6	240,000	270	278,000	10,900	< 20	49,000	130
Irrigation water:	12	1.3×10^6	13×10^6	7.9×10^6	--	--	--	330,000	7.0×10^6	2.3x10
Farm No. 12 - 1963 Study. Irrigation Source: Fulton Branch										
Vegetables:										
Turnips	4	180,000	2.12×10^6	440,000	80	542,000	33,000	20	70,000	4,900
Soil:	3	920,000	4.9×10^6	940,000	79,000	4.9×10^6	2.47×10^6	330	1.3×10^6	23,000
Irrigation Water:	12	2.78×10^6	17.2×10^6	10.9×10^6	--	--	--	630,000	17.2×10^6	2.3x10
Farm No. 12 - 1964 Study. Irrigation Source: Fulton Branch										
Vegetables:										
Cabbages - Market	5	78	64,000	4,600	< 18	17,000	350	< 18	790	< 18
Soil:	6	17,000	220×10^6	590,000	1,400	200×10^6	33,000	20	3.3×10^6	4,000
Irrigation Water:	10	49,000	70×10^6	3.3×10^6	49,000	70×10^6	1.5×10^6	7,000	7.9×10^6	410,000

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 13 - 1963 Study. Irrigation Source: Fulton Branch										
Vegetables: Cabbage	5	70	920,000	10,900	< 20	10,900	50	< 20	< 20	< 20
Soil:	4	3,500	130,000	66,000	< 20	70,000	700	< 20	7,900	< 20
Irrigation Water:	11	2.78×10^6	17.2×10^6	10.9×10^6	--	--	--	630,000	17.2×10^6	2.3×10^6
Farm No. 14 - 1964 Study. Irrigation Source: Left Hand Ditch										
Vegetables: Leaf Lettuce	5	20	70×10^6	45,000	< 18	4.9×10^6	330	< 18	330	< 18
Carrots - Field	1	--	--	280,000	--	--	1,800	--	--	20
Soil:	3	2,800	33×10^6	9,500	61	2.8×10^6	78	20	110	20
Irrigation Water:	7	15	49,000	4,800	12	49,000	2,100	4.6	49,000	430
Farm No. 15 - 1964 Study. Irrigation Source: Left Hand Ditch										
Vegetables: Leaf Lettuce	3	350,000	35×10^6	$>1.6 \times 10^6$	2,300	7.0×10^6	1.6×10^6	2,300	920,000	11,000
Radishes - Field	1	--	--	16×10^6	--	--	--	--	--	130,000
Soil:	3	180,000	1.2×10^6	690,000	17,000	280,000	148,500	45	1,300	670
Irrigation Water:	4	15	49,000	7,200	12	49,000	4,800	4.6	49,000	1,200

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 16 - 1964 Study. Irrigation Source: Left Hand Ditch										
Vegetables:										
Leaf Lettuce	8	93	33×10^6	4,900	< 18	7,900	510	< 18	7,900	90
Carrots - Field	1	--	--	79,000	--	--	17,000	--	--	< 18
Soil:	11	790	3.3×10^6	40,600	< 18	2,300	444	< 18	< 18	< 18
Irrigation Water:	11	15	49,000	4,800	12	49,000	2,100	4.6	49,000	230
Farm No. 17 - 1964 Study. Irrigation Source: Niwot Ditch										
Vegetables:										
Cabbage - Field	3	< 18	460	18	< 18	20	< 18	< 18	20	< 18
Cabbage - Market	8	45	170,000	9,800	< 18	9,200	19	< 18	< 18	< 18
Soil:	6	61	33,000	8,800	< 18	11,000	162	< 18	1,700	780
Irrigation Water:	16	230	49,000	7,900	230	13,000	3,300	110	7,900	790
Farm No. 18 - 1964 Study. Irrigation Source: Swede Ditch										
Vegetables:										
Leaf Lettuce	5	700	220,000	3,300	110	49,000	700	40	4,900	69
Radishes - Field	2	23×10^6	35×10^6	29×10^6	540,000	4.8×10^6	2.67×10^6	18,000	22,000	20,000
Carrots - Field	1	22	--	110,000	--	--	3,600	--	--	130

BACTERIAL DENSITIES OF VEGETABLES, SOILS, AND IRRIGATION WATER

Type of Sample	No. of Samples	COLIFORM MPN								
		Confirmed			Completed			Fecal		
		Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median
Farm No. 18 - 1964 Study (cont'd)										
Soil:	6	13,000	7.9×10^6	49,000	2,200	1.4×10^6	5,300	240	700,000	820
Irrigation Water:	13	1,100	70,000	7,900	1,100	13,000	4,900	130	4,900	790
Farm No. 19 - 1964 Study. Irrigation Source: Rough & Ready										
Vegetables:										
Carrots - Market	4	1,400	230,000	12,200	< 18	330	17	< 18	20	< 18
Soil:	5	140	2.1×10^6	3,500	< 18	2,200	130	< 18	68	< 18
Irrigation Water:	12	44	17,000	1,600	33	3,300	870	11	1,100	230

APPENDIX D

Chemical Data of Irrigation Water According to Farm and Source

CHEMICAL DATA[1/] OF IRRIGATION WATER ACCORDING TO FARM AND SOURCE

Farm Number and Irrigation Water Source	Number of Samples	Temperature °C	pH	DO (mg/l)	BOD (mg/l)	Conductivity (umhos/cm)
Farm No. 1 - 1963 Study Seepage from Hodgson and Warrior Ditch	17	16.0	7.4	--	--	--
Farm No. 2 - 1964 Study Burlington Ditch	33	20.5	7.4	1.8	38	1010
Farm No. 3 - 1963 Study Gardeners Ditch	23	23.5	7.2	--	--	--
Farm No. 4 - 1963 Study Gardeners Ditch	23	23.5	7.2	--	--	--
Farm No. 5 - 1964 Study Gardeners Ditch	11	23.5	7.3	--	--	--
Farm No. 5 - 1963 Study Gardeners Ditch	23	23.5	7.2	--	--	--
Farm No. 6 - 1964 Study Gardeners Ditch	18	21.0	7.5	1.2	24	823
Farm No. 7 - 1963 Study Duggan Ditch	9	27.5	7.4	--	--	--
Farm No. 7 - 1964 Study Duggan Ditch	8	21.0	7.5	0.0	29	929
Farm No. 8 - 1964 Study Duggan Ditch	8	22.0	7.5	1.4	25	868

1/ All Data are Median Values

Chemical Data of Irrigation Water According to Farm and Source

Farm Number and Irrigation Water Source	Number of Samples	Temperature °C	pH	DO (mg/l)	BOD (mg/l)	Conductivity (umhos/cm)
Farm No. 9 - 1964 Study Burlington Ditch	11	18.5	7.6	0.0	63	1110
Farm No. 10 - 1964 Study Fulton Ditch	9	19.0	7.5	0.4	31	890
Farm No. 11 - 1963 Study Brantner Ditch	10	21.0	7.5	--	--	--
Farm No. 12 - 1963 Study Fulton Branch	11	21.0	7.4	--	--	--
Farm No. 12 - 1964 Study Fulton Branch	10	17.8	7.5	2.3	38	965
Farm No. 13 - 1963 Study Fulton Branch	11	21.0	7.4	--	--	--
Farm No. 14 - 1964 Study Left Hand Ditch	11	16.5	7.6	8.4	1	60
Farm No. 15 - 1964 Study Left Hand Ditch	11	16.5	7.6	8.4	1	60
Farm No. 16 - 1964 Study Left Hand Ditch	11	16.5	7.6	8.4	1	60
Farm No. 17 - 1964 Study Niwot Ditch	16	16.5	8.1	8.2	1.5	773

Chemical Data of Irrigation Water According to Farm and Source

Farm Number and Irrigation Water Source	Number of Samples	Temperature °C	pH	DO (mg/l)	BOD (mg/l)	Conductivity (umhos/cm)
Farm No. 18 - 1964 Study						
Swede Ditch	13	13.5	8.3	9.4	1.3	194
Farm No. 19 - 1964 Study						
Rough & Ready	12	17.8	8.6	8.8	2.4	549